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The Role of Coenzyme Q in Cellular Metabolism: Current Biological and Clinical Aspects

Guest Editor

Richard H. Haas

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Special Issue: The Role of Coenzyme Q in Cellular Metabolism: Current Biological and Clinical Aspects

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Foreword

This special edition of 'Mitochondrion' has provided a unique opportunity to gather together many of the world's experts on Coenzyme Q who have graciously provided timely reviews of their respective areas of research expertise. I am particularly indebted to Professors Frederick Crane and Anthony Linnane for their support, helpful advice on the composition of this edition and for providing excellent manuscripts of their own. Professor Crane's article is appropriately the first in this collection as he is the discoverer of ubiquinone. He provides an invaluable historical perspective along with an overview of the current state of research. Professor Linnane adds a cautionary note pointing out that the pro-oxidant and signaling role of Coenzyme Q is critical for an array of cellular functions and should not be overlooked in the current focus on antioxidant function and high dose treatment.

The timing of this special edition is opportune. Large scale phase 3 clinical trials of high dose Coenzyme Q_{10} in neurodegenerative disease are about to start and just in the last year the first molecular biochemical defects underlying the childhood primary Coenzyme Q diseases have been reported. As the table of contents details we start with Professor Crane's historical perspective followed by superb reviews of the mitochondrial and cellular role of Coenzyme Q progressing to the antioxidant role, pro-oxidant role, cellular synthetic pathway and the role in aging, oxidative stress and human disease.

Novel designer quinone derivatives targeted to the mitochondrion are discussed as promising therapeutic agents. The therapeutic role is further explored with practical information on pharmacokinetics, bioavailability and assay methodologies moving on to disease descriptions and the evidence for treatment efficacy in the rare genetic disorders of primary Coenzyme Q_{10} deficiency, Friedreich's ataxia, mitochondrial disease, phenylketonuria and the common neurodegenerative disorders of aging. A discussion of the evidence supporting the use of supplementation in cardiovascular disease is complimented by an overview of statin drug effects on the mevalonic pathway and Coenzyme Q_{10} synthesis.

This edition would not have been possible without the rapid and thoughtful review of these contributions by expert reviewers from around the world to whom I owe a particular debt of gratitude. The goal of this edition is to provide a state of the art comprehensive review of a unique molecule which as its original name ubiquinone implies is found ubiquitously throughout the cell. It is hoped that the reader will find this a useful reference volume for many years.

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ELSEVIER

Discovery of ubiquinone (coenzyme Q) and an overview of function

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Abstract

Details of the discovery of ubiquinone (coenzyme Q) are described in the context of research on mitochondria in the early 1950s. The importance of the research environment created by David E. Green to the recognition of the compound and its role in mitochondria is emphasized as well as the dedicated work of Karl Folkers to find the medical and nutritional significance. The development of diverse functions of the quinone from electron carrier and proton carrier in mitochondria to proton transport in other membranes and uncoupling protein control as well as antioxidant and prooxidant functions is introduced. The successful application in medicine points the way for future development.
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The discovery of coenzyme Q was not a simple accident as sometimes mentioned. It was the result of a long train of investigation into the mechanism of, and compounds involved in biological energy conversion. Its origin can be traced back to the early studies of biological oxidation especially those of Warburg and Keilin which introduced a chain of cytochromes as electron carriers to oxygen. These studies were extended by Chance (1954) with the development of the rapid flow-dual beam spectrophotometer. With this instrument the rate of reduction of the component flavins and cytochromes could be measured which led to definition of an electron transport chain from NADH or succinate through flavin, cytochrome *b*, cytochrome *a*, cytochrome *c*, cytochrome *a* and *a*₃ to oxygen.

The next question was how these cytochromes were associated and how they drove the formation of ATP.

In the early 1950s good biochemistry required the purification of enzymes for proper definition of their catalytic properties. When David Green proposed that the citric cycle and oxidative phosphorylation was contained in an organized complex which he called cyclophorase it was met by extreme skepticism (Green et al., 1948). The development of microscopic staining and ultracentrifuge techniques to identify the respiratory particles as mitochondria has been reviewed by Lehninger (1964). In 1950 Green at the Enzyme Institute, University of Wisconsin then embarked on a major program to determine how the enzymes of the fatty acid oxidation and citric cycle oxidation were organized and how this contributed to energy coupling in oxidative phosphorylation. A unique and crucial component of this program was isolation of large amounts of mitochondria. Most laboratories involved in these studies used small amounts of rat liver or pigeon breast muscle mitochondria which gave good ratios of ATP formed to oxygen consumed (P/O ratios) approaching 3 but left little to work with in enzyme isolation.

To obtain a large amount of material to work with Green arranged to get up to a dozen beef hearts per day from the Oscar Meyer plant in Madison. These hearts were homogenized in a large blender followed by centrifugation in a large 13 l centrifuge to remove unwanted material such as myosin. The supernatant was then centrifuged in a large industrial size sharples machine to sediment the mitochondria as a brown paste which were suspended in a sucrose phosphate buffer prior to freezing. The production was

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80–100 g of mitochondrial protein per day. To start a study we could just go to the freezer and take out a liter of concentrated mitochondria.

With a good supply of mitochondria available Green instituted a program to systematically separate parts of the electron transport system to see how they interacted and how this interaction was related to ATP formation. By using aqueous extractions it was possible to prepare isolated flavoproteins, succinc dehydrogenase and NADH dehydrogenase, which could react with artificial electron acceptors but not with cytochrome c. Further fractionation required the use of detergents. The first success was separation of a succinate cytochrome c reductase preparation which contained flavin, cytochrome b and cytochrome $c1$ by Green and Burkhard (1961). In the meantime Wainio et al. (1948) using deoxycholate had succeeded in separating a cytochrome c oxidase which contained only cytochromes a and a_3. This led to studies with cholate detergents which gave other fractions most notably a NADH cytochrome c reductase. Further fractionation led to loss of activity. Until the discovery of coenzyme Q made it possible for Hatefi et al. (1962) to reconstruct the complete electron transport system. During these fractionation studies it became clear that the lipid in the membrane was closely associated with the cytochromes and flavine. According to theories of membrane structure at that time (late 1950s) the components of the electron transport would be bound to the surfaces of the mitochondrial lipid bilayer. Since the products of fractionation all retained lipid it became apparent that the association of the carrier protein and lipid was very strong. This led to a consideration of the possible functional role of lipid. At this point I felt that detergent based fractionation of the mitochondrial membranes had been exploited as much as possible so Carl Widmer and I started a study of the lipids associated with the various fractions. At about this time (1955) Nason and coworkers (Donaldson et al., 1958) reported inducing a requirement for α-tocopherol in mitochondrial electron transport by isooctane extraction. I tried the effect of isooctane on mitochondrial electron transport and found isooctane induced inhibition could be reversed by α-tocopherol but that the reversal could also be achieved with beef serum albumin. I wrote to Nason and he agreed that serum albumin had something that reversed the isooctane inhibition. These experiments led to two considerations: first that the electron transport proteins were remarkably resistant to denaturation by hydrocarbon solvents which laid the ground work for the use of these solvents to extract coenzyme Q in studies of its activity. Second it led me to consider if any other vitamin might be needed in the electron transport, loss of which might account for fractions where activity could not be restored. To see what vitamins might be involved I sent a liter of mitochondria to the Wisconsin Alumni Research Laboratory for vitamin analysis. Several B vitamins were found in the beef heart mitochondria preparation, but no niacin. A significant amount of α-tocopherol was present. In separate assays I found no vitamin K. The research laboratory did not do vitamin A analysis so I set out to do that. Because of my plant physiology background I was interested in plant mitochondria. Since the Enzyme Institute was uniquely equipped for study of mitochondria I took advantage of the facilities to make cauliflower mitochondria on the weekends when the laboratory was essentially empty (Crane, 1957). Surprisingly the cauliflower mitochondria were yellow, not brown like animal mitochondria. So the thought occurred to me that the cauliflower mitochondria might contain carotenoids and that beef heart mitochondria might have carotenoids hidden under the brown pigments. I figured that many compounds with absorption spectra in the visible range had loose electrons and could act as electron carriers in oxidation reduction reactions so carotenoids were possible carriers. It turned out that beef heart mitochondria have carotenoids but no vitamin A. If the amount of carotenoids is estimated as β carotene by spectral absorption of unsaponifiable lipid at 448 nm the 9 nmole/mg protein found is higher than the concentration of cytochromes (0.8–0.4 nmole/mg protein) so it would be sufficient to function in electron transport. The lack of vitamin A was confirmed by a negative Carr–Price reaction. When the non-saponifiable lipid was chromatographed on an alumina or Decalso column three carotenoid bands were eluted with heptane. Following the carotenoids was a broad yellow band which eluted off the column with 2% ethyl ether in heptane. This compound had a broad absorption peak around 400 nm and was first observed December 3, 1956. Later it was found to have a strong peak at 275 nm. To guard against saponification artifacts we prepared the "400" compound from a petroleum ether–ethanol extract of mitochondria. Removal of the solvent from the "400" fraction eluate left a yellow oil. When the oil was dissolved in ethanol and put in the refrigerator for 2 days long needle like yellow crystals formed which could be filtered off. These were recrystalized until a constant melting point of 48–49 °C was maintained (Crane et al., 1957). The next question was what was this strange compound and could it have a role in electron transport? We knew that it was probably not a short chain carotenoid (like colorless vitamin A) or a long chain carotenoid (like β carotene) because of lack of multiple peaks in the visible spectra (400 nm) and lack of Carr Price reaction (40 carbon poly unsaturated carotenes like β carotene show Carr Price peaks in the infrared region $\sim$ 1000 nm). In the organic chemistry course at the University of Michigan Prof Bachman taught organic chromophore which included quinones. As a graduate student in plant physiology I was exposed to discussion of possible roles of quinones in plant respiration which was not expected in animals because benzoquinones were reported to be rare in animals (Thomson, 1957). Bob Lester and I discussed the idea that the 400 compound might be a quinone. We dug a small book by Morton on organic spectra out of the library and found the spectrum of benzoquinone with an oxidized peak at 254 nm and a smaller peak in the

reduced state at 290 nm. When we refluxed the 400 compound with ascorbic acid and HCl the 275 band shifted to a smaller peak at 290 nm which was consistent with formation of a hydroquinone. Joe Hatefi suggested using borohydride as an easier reducing agent for direct reduction in ethanol. The evidence that the 400–275 compound was a quinone led to consideration of substitutions on the ring to modify the quinone spectra to shift the peak to 275 nm. Since it appeared to be a quinone with an absorption peak at 275 nm we started to call it Q275.

Further analysis found two methoxy groups and isoprene units on the quinone. Molecular weight determination by boiling point depression gave variable results but redox titration of the reduced quinone gave a molecular weight between 797 and 920. Subsequent molecular weight determination by X-ray diffraction gave 849 (Lester et al., 1959). We also developed an assay for Q275 function in succinoxidase by extraction of beef heart mitochondria with heptane to remove some of the coenzyme Q. This extraction decreased succinate oxidase activity which could be restored by adding back Q275. The carotenoids from mitochondria, vitamin A, α-tocopherol and α-tocopherol quinone did not restore activity after heptane extraction but surprisingly cytochrome *c* did. Later studies using other solvents for extraction showed a requirement for Q275 for cytochrome *c* reduction and both Q275 and cytochrome *c* for succinic oxidase (Green, 1961). We also found that Q275 was reduced when incubated with mitochondria and that the quinol was oxidized by mitochondria. These oxidation and reduction changes were inhibited by specific mitochondrial inhibitors. The evidence clearly indicated that Q275 could be a component in the mitochondrial electron transport. In April 1957, we sent a short note for publication in BBA (Crane et al., 1957) outlining our evidence for the role of the quinone in mitochondria. Following the publication Karl Folkers at Merck, Sharpe, and Dohm called Green to arrange a collaboration in the study of quinone. Folkers felt that if the quinone was essential for mitochondrial electron transport it was likely that in some people deficiencies could occur which would mean that the quinone could be a vitamin. Since there was no vitamin Q at that time Folkers thought that a functional designation, coenzyme Q, would be a good tentative name until a vitamin Q function could be established. This was the beginning of a dedicated 10 year search by Folkers for nutritional significance culminated by Yamamura's use in the treatment of heart failure in 1967 (Yamamura, 1977). Folkers's efforts resulted in the organization and publication of a series of symposia on clinical and medical applications of coenzyme Q (Folkers and Yamamura, 1981, 1984, 1986; Yamamura et al., 1980; Folkers et al., 1991; Littarru et al., 1994, 1997). These symposia have been continued by the International Coenzyme Q10 Association (Biofactors, 1999, 2003).

To determine the specificity of mitochondrial electron transport for coenzyme Q the Merck group synthesized all combinations of dimethoxy benzoquinones and we found that only the 2,3 dimethoxy quinone analog could restore succinoxidase after extraction of coenzyme Q.

The essential role of coenzyme Q in mitochondrial electron transport was questioned on the basis that the rate of oxidation reduction was slower than the other electron carriers such as cytochrome (Redfearn, 1961). This problem was solved by Klingenberg's (1968) consideration that there was 10 times as much coenzyme Q as other carriers. Therefore reduction of each molecule of Q would be slower than the less abundant carriers.

The groundwork for broader functions of coenzyme Q in membranes other than mitochondria was laid at this time when Ramasarma and coworkers (Sastry et al., 1961) showed that coenzyme Q was in other cellular membranes. This observation has led to consideration of coenzyme Q as an antioxidant and as a carrier for proton transfer across other membranes.

At the same time that we were looking for vitamin A in mitochondria and finding Q275 Morton's laboratory in Liverpool were studying a compound (SA) which increased in vitamin A deficient rat liver. They were interested in the possible relation between sterols and vitamin A so they first considered SA to be an ene dione steroid. When we found the spectrum they published for SA was the same as for Q275 Bob Lester and I wrote to Morton to say we thought it was a quinone. It was nice to find out that they agreed with us. This indicates how the framework of the research determines the interpretation of the observation. The work on SA has been discussed by Morton (1961).

By investigating other plant and animal material we supported an almost universal role of coenzyme Q. A similar conclusion was reached by Morton's group which led to the name ubiquinone. Variation was found in the length of the isoprenoid side chain which ranged from 10 to 6 isoprenoid groups. In addition, we found in green plants and chloroplasts a similar quinone with absorption maximum at 254 nm which we named plastoquinone. It later was shown to be essential for photosynthesis with a role in chloroplasts like coenzyme Q in mitochondria (Bishop, 1961).

The finding of an essential quinone in mitochondria naturally led to consideration of a quinone derivative as an intermediate in oxidative phosphorylation. It was generally considered that a phosphorylated intermediate would be activated by oxidation to phosphorylate ADP to make ATP. Various quinone derivatives, especially chromanols, were tested without success. It took a different frame of reference introduced by Peter Mitchell to find how coenzyme Q contributed to ATP synthesis (Mitchell, 1975). Mitchell had been considering formation of a membrane potential as a driving force for ATP synthesis. If the oxidation and reduction of coenzyme was oriented across the membrane it would provide a way to generate a membrane potential by proton gradient generation across the membrane. When coenzyme Q is reduced it takes up two protons which are released when it is oxidized. Thus the energy conversion role of coenzyme Q was in the protonation and not in the electron transport function. Protons are taken up inside

the mitochondrial membrane and released outside as the quinone oscillates back and forth. Thus the unique role for coenzyme Q in energy conversion was discovered.

Folkers search for a coenzyme Q deficiency condition ranged from tests on kwashiorkor to dystrophy. These early investigations were hampered initially by supply of sufficient coenzyme Q for large scale testing and poor uptake when administered in crystalline form. In early studies analogs which were easier to synthesize such as coenzyme Q_7 and hexahydro Q_4 were tested without great success. The supply problem was solved by production through yeast fermentation by Kanegafuchi Chemical Co and by chemical synthesis by Nisshin Chemicals Co. Pharmaceutical preparations were made available by Eisai Co in 1974 (Folkers, 1985).

It took some time before poor absorption of orally administered coenzyme Q was measured. It was found that only 2–3% of crystalline coenzyme Q was taken up in the blood. In hindsight it is clear that a combination of low dosage, 30–60 mg per day, and poor absorption was unlikely to be very effective in treatment of any deficiency. The first break came with the demonstration that absorption was remarkably improved by taking coenzyme Q mixed with peanut butter. Further study of absorption led to development of gel capsules which gave a high percentage of uptake and led to significant increase in coenzyme Q in the blood (Bhagavan and Chopra, 2006). There still remains a question of what controls uptake into various tissues. Further study of control of breakdown and excretion is needed. For many years the focus of coenzyme Q research was on its role in energy transduction in mitochondria. It gradually became recognized that it was widely distributed in cell membranes and could play a role in antioxidant function and proton transport in other membranes. Early study by Ramasarma and coworkers (Sastry et al., 1961) found coenzyme Q in microsomes as well as mitochondria. Later we (Crane and Morre, 1977) found a high concentration of coenzyme Q in Golgi membranes and found that it functioned in a non-mitochondrial electron transport. Further study by Dallners group (Ernster and Dallner, 1995) showed coenzyme Q in all endomembranes. The finding of coenzyme Q in all membranes brought on a concept of coenzyme Q as an important antioxidant (Kagan et al., 1990). Early studies had shown that coenzyme Q hydroquinone is an excellent free radical scavenging antioxidant but its role was restricted to mitochondria until a general membrane distribution was shown. Even greater significance was apparent when it was shown that reduced coenzyme Q could restore antioxidant function to oxidized tocopherol. This is important because endomembranes have enzymes that can reduce coenzyme Q but none for reduction of oxidized tocopherol directly. On the other hand a role of hydroquinone as an oxygen radical generator is being explored (Crane, 2000). The formation of a semiquinone radical during the oxidation–reduction in mitochondria was observed soon after discovery of coenzyme Q and has been indicated as a basis

for part of the peroxide production in mitochondria when ADP is not available for phosphorylation or inhibition of Q oxidation are present (antimycin) (Ozawa, 1985). During oxidoreduction in other membranes coenzyme Q may form a partially reduced semiquinone radical. If the semiquinone reacts with oxygen the superoxide radical can be formed which can be converted to hydrogen peroxide. Thus coenzyme Q could contribute to the generation of reactive oxygen radicals which might contribute to destruction of membrane lipids or be responsible for hydrogen peroxide signaling (Rhee, 2006). Further significance of the presence of coenzyme Q in endomembranes comes from the evidence that it functions as a proton transferring redox agent in acidification of lysosomes. This is an area which needs further study because it may relate to proton transfer across the plasma membrane also (Crane, 2000). Other effects of coenzyme Q which have not been fully examined are effects on membrane fluidity (Turunen et al., 2004) and on phospholipid metabolism. The recent finding that coenzyme Q is required for the protonophoric mitochondrial uncoupling protein indicates further unknown role in control of cell metabolism (Echtay et al., 2000). Thus the all out attack which Green developed on mitochondrial electron transport led to an unexpected molecule with diverse biochemical significance. The full extent of its diversity remains to be explored. One can speculate that Morton in his study of vitamin A relation to ubiquinone would have developed the antioxidant approach which would have led back to mitochondria as a prime basis of superoxide removal or production. The later years of coenzyme Q history revolve around Karl Folkers and his search for a vitamin-like requirement for coenzyme Q which led to therapeutic approaches. For 40 years from 1958 to 2000 he organized an unrelenting campaign to find nutritional and therapeutic significance for coenzyme Q. After determining the structure by synthesis of coenzyme Q he was instrumental in organizing the CIBA Symposium on Quinones in Electron Transport which brought up the general role of lipophilic quinones in biological electron transport in mitochondria, chloroplasts, and bacteria. Folkers then did pioneering studies to try and ameliorate diseases such as kwashiorkor and dystrophy without much success. He also organized a series of six synopsia on coenzyme Q from 1977 to 1990 which brought together workers in both basic biochemistry and medical applications. The first successful application of coenzyme Q to a medical problem was in Yamamura's treatment of congestive heart failure. The search for medical or nutritional application continues and the coenzyme Q synopsia continue under the International Coenzyme Q association. The medical conditions which have shown some response to coenzyme Q (Ebadi et al., 2001) now include congestive heart failure (Langsjoen and Langsjoen, 1998), Immune deficiency (Bliznakov and Hunt, 1987), Encephalomyopathy or ataxia (Quinzii et al., 2006), Parkinsonism and Huntingtons disease (Shults, 2003; Beal, 2004; Ryu and Ferrante, 2005), and Cancer (Hodges et al., 1999; Brea-Calvo et al., 2006).

Indication of therapeutic effects in diabetes (Hodgson et al., 2002) and relief of statin side effects (Littarru and Tiano, 2005) have also been reported. Thus Folkers long search has been more productive than expected and has developed from more functions of coenzyme Q than simple protonophoric electron transport. The biochemical pathways for coenzyme Q synthesis were investigated soon after the discovery (Clarke, 2000). In general all animals, plants, and bacteria synthesize their own coenzyme Q so a typical vitamin deficiency was not found until recently. Now several instances of mitochondrial deficiency disease have been related to coenzyme Q (Di Mauro, 2004). Knowledge of the biochemical synthesis now permits a molecular biology approach to deficiency in synthesis. A mutation in a gene necessary for coenzyme Q synthesis has recently been found to explain a deficiency (Quinzii et al., 2006).

In contrast to its functions as an electron transport protonophore and antioxidant there are conditions under which quinol can generate superoxide as an oxygen radical. Under conditions which lead to increased coenzyme Q reduction in mitochondria it is possible to show increased oxygen radical generation (Nohl et al., 1996). Since reduced Q is also found in other membranes and especially in blood plasma it may be involved in oxygen radical generation at these sites also. The generation of superoxide and hydrogen peroxide may also be a basis for activation of defensive genes to protect against free radical damage and aging (Linnane and Eastwood, 2004).

True there are many questions about coenzyme Q in medicine, nutrition, and basic biochemistry which remain to be investigated (Dhanasekaren and Ren, 2005). Although the role in electron transport and proton transport for energy conversion are quite well understood more study is needed about antioxidant–prooxidant balance, mechanism of uncoupled protein action, and control of membrane fluidity (Fato et al., 1984).

To gain a basis for understanding requirements for coenzyme Q a better understanding of genetic and nutritional control of serum and tissue levels of coenzyme Q and excretion of coenzyme Q is needed. To further the understanding of requirements for coenzyme Q a more readily available clinical assay of coenzyme Q would be desirable since the extent and basis for variation in serum and tissue levels is not known.

Acknowledgements

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The role of Coenzyme Q in mitochondrial electron transport

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Abstract

In mitochondria, most Coenzyme Q is free in the lipid bilayer; the question as to whether tightly bound, non-exchangeable Coenzyme Q molecules exist in mitochondrial complexes is still an open question.

We review the mechanism of inter-complex electron transfer mediated by ubiquinone and discuss the kinetic consequences of the supramolecular organization of the respiratory complexes (randomly dispersed vs. super-complexes) in terms of Coenzyme Q pool behavior vs. metabolic channeling, respectively, both in physiological and in some pathological conditions. As an example of intra-complex electron transfer, we discuss in particular Complex I, a topic that is still under active investigation.
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1. Introduction

The natural Coenzyme Q (CoQ, ubiquinone) is 2,3-dimethoxy-5-methyl-6-polyprenyl-1,4-benzoquinone, where the polyprenylated side chain is 6–10 units long. Within mammals, only CoQ_9 and CoQ_{10} are found, with CoQ_9 only distributed among rodents. CoQ exists in three redox states, fully oxidized (ubiquinone), semiquinone (ubisemiquinone), and fully reduced (ubiquinol); nevertheless, the existence of different possible levels of protonation increases the possible redox forms of the quinone ring (Kozlov et al., 1998). Due to its extreme hydrophobicity, natural CoQ can be present in three physical states only: forming micellar aggregates, dissolved in lipid bilayers, and bound to proteins. The former state is very important when working with CoQ in cell-free systems (Fato et al., 1986), however in the living cell CoQ should be distributed among the other two states.

How much CoQ is protein-bound? If we consider bound CoQ as stoichiometric with one site in the complexes that have been shown to contain bound CoQ (I, II, III) (Vinog-radov et al., 1995; Lee et al., 1995; Trumpower, 1981), in beef heart mitochondria we come up to no more than 0.35 nmol/mg protein, that would increase to ca 0.5 nmol assuming more than one site to be fully occupied in Complex I and Complex III. Since the total CoQ content is higher than 3 nmol/mg protein (Capaldi, 1982; Fato et al., 1996), we must assume that most CoQ (>84%) is free in the bilayer. A direct study (Lass and Sohal, 1999) of the amount of CoQ bound to mitochondrial proteins in five different mammalian species has shown values between 10% and 32% of total CoQ to be protein-bound.

It has been assumed for long time that the shape of the CoQ molecule is linear, with some possibility of rotation allowed for the long isoprenoid tail. Bending of the molecule is required in a model proposed by us (Lenaz, 1988), on the basis of previous evidence and of theoretical considerations, and confirmed by linear dichroism studies (Samorì et al., 1992) of the location of CoQ_{10} in the hydrophobic mid-plane of the lipid bilayer, with the polar head oscillating about the third isoprene unit between the mid-plane (wholly linear shape) and the polar heads of the phospholipids (maximal bending of 90°). The model allows for movement of the redox centre of CoQ, that is required for interaction with other redox centres in the mitochondrial complexes.

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For example, the Rieske iron–sulphur centre of Complex III, that is the first electron acceptor from ubiquinol, is situated at the level of the hydrophilic heads of the phospholipids (Xia et al., 1997) and iron–sulphur cluster N2 of Complex I, that is considered as the electron donor to CoQ, is situated at the interface between the hydrophilic and hydrophobic moieties of the enzyme. The model also allows for the reduced form, in which the benzoquinone ring is more polar, to be preferentially located at the polar surface of the membrane (Lenaz et al., 1992).

Contrary to these predictions, a study of molecular dynamics computer simulation of CoQ homologues in the vacuum starting from different initial configurations has shown that the conformation with lowest energy level is a folded one, where the polar head is in tight contact with the last isoprenoid unit of the hydrophobic tail (Di Bernardo et al., 1998). Within the series of homologues, the cut-off for the folded conformation is four isoprenoid units. The folded conformation was found for both oxidized and reduced quinones, however only small energy differences were found between oxidized and reduced ubiquinones in the folded conformation. The difference is not the same for the various quinones: the different behaviour would be the result of geometric differences imposed by the folding of isoprenoid units of slightly different length. For example the enthalpy difference between oxidized and reduced form is much higher for CoQ_{10} than for CoQ_9, indicating that the former has a stronger tendency to become oxidized, i.e. a lower midpoint redox potential (unpublished observations from our laboratory, cf Lenaz, 2001).

Although the molecular modelling has been performed in a vacuum, we have reason to believe that the folded conformations also apply to the CoQ homologues in natural membranes; this idea is supported by the experimental demonstration by magnetic resonance techniques that ubisemiquinones are folded in organic solvents (Joela et al., 1997); moreover, apparently inexplicable differences found in the antioxidant behaviour of CoQ_9 and CoQ_{10} (Matsura et al., 1992; Edlund et al., 1994; Lass et al., 1997) might provide an explanation in their steric configuration.

There are important implications of a folded structure. First, the similar size of short and long homologues would explain the similar high rates of lateral diffusion found in our laboratory for all quinone homologues (Fato et al., 1986; Di Bernardo et al., 1998). In addition, protein binding during electron transfer may require unfolding, contributing to the high activation energy and low collision efficiency observed for electron transfer (e.g. Fato et al., 1993; Fato et al., 1996).

2. Inter-complex electron transfer mediated by CoQ

2.1. Complexes involved as partners in CoQ-mediated electron transfer

The electron transfer chain consists of four major multisubunit complexes designated as NADH-CoQ reductase (Complex I), succinate-CoQ reductase (Complex II), ubiquinol–cytochrome c reductase (Complex III) and cytochrome c oxidase (Complex IV). The best fit unit stoichiometry between complexes in beef heart mitochondria is 1, Complex I; 1.3, Complex II; 3, Complex III; 6.7 Complex IV (Schägger and Pfeiffer, 2001). In addition there are 0.5 ATP synthase (also called Complex V) and 3–5 U of the ADP/ATP translocase (catalyzing the equimolar exchange of ADP and ATP across the inner membrane) for each cytochrome oxidase, and there is one $NADH/NADP^+$ transhydrogenase per Complex I (Capaldi, 1982). Indeed, wide differences in cytochromes, Coenzyme Q and pyridine nucleotide contents of mitochondria from different species, as well as from different organs of the same species, have been reported (Battino et al., 1990; Blair et al., 1963; Green and Wharton, 1963; King et al., 1964); data in the literature indicate that even the molar ratios of the respiratory components vary significantly.

The inner membrane contains several other proteins having electron transfer activity in smaller amounts (Ernster and Schatz, 1981); among these there are electron transfer flavoproteins capable of feeding electrons to the respiratory chain by pathways not involving Complex I and/or NAD^+, i.e. glycerol-3-phosphate dehydrogenase (Klingenberg, 1970), electron transfer flavoprotein (ETF)-ubiquinone oxidoreductase (Frerman, 1990), dihydroorotate dehydrogenase (Jones, 1980), choline dehydrogenase (Lin and Wu, 1986), besides alternative NADH dehydrogenases (Kerscher, 2000) in mitochondria from several organisms, especially plants and fungi. Moreover, alternative or branched pathways of electron transfer also occur, departing from CoQ: these are the alternative ubiquinol oxidases from bacteria (Musser et al., 1993) and plant and fungi mitochondria (Berthold et al., 2000).

Here, we discuss the enzymes involved in electron transfer to and from CoQ.

2.1.1. Complex I

With the notable exception of *Saccharomyces cerevisiae*, most organisms possess Complex I, a very large enzyme catalyzing the first step of the mitochondrial electron transport chain (Saraste, 1999; Schultz and Chan, 2001). The enzyme oxidizes NADH in the mitochondrial matrix and reduces CoQ in the lipid bilayer of the inner mitochondrial membrane. The total number of subunits in the bovine enzyme is 46 (Carroll et al., 2003) or 45 (Carroll et al., 2006) for a molecular mass of about 1000 kDa. Seven subunits are the products of the mitochondrial genome (Chomyn et al., 1985, 1986) and correspond to hydrophobic components named ND1-ND6 and ND4L. The minimal active form of the enzyme is that found in bacteria, composed of 14 subunits, all of which are homologous to their mitochondrial counterparts, while all other "accessory" subunits still have an undefined role. From structural and phylogenetic considerations, the enzyme is envisaged to consist of three different sectors: a *dehydrogenase* unit and a *hydrogenase*-like unit, constituting the peripheral

arm protruding into the matrix, and a *transporter* unit deeply embedded in the membrane and involved in proton translocation (Friedrich and Scheide, 2000; Mathiesen and Hägerhäll, 2002; Friedrich and Bottcher, 2004). Fig. 1 shows a general scheme illustrating the electron transfer pathway from NADH to CoQ; also two major sites for reduction of the artificial acceptor 2,4-dichloro-phenol-indophenol (DCIP) are shown.

Several prosthetic groups contribute to electron transfer within the enzyme: FMN is the entry point for electrons, that are then transferred to a series of iron–sulphur clusters (Ohnishi et al., 1998). Enzymes from different sources have different numbers of iron–sulphur clusters, most of which share the same midpoint potential. Two clusters present different characteristics: N1a, with a structure Fe_2S_2, has the lowest midpoint potential ($E_m = -370$ mV), while N2, with the structure Fe_4S_4 resides at the interface between the PSST and the 49 kDa subunits (Kerscher et al., 2001b), has the highest midpoint potential (E_m between -150 mV and -50 mV), presenting EPR magnetic interactions with the ubisemiquinone radicals; for these reasons it is considered to be the direct electron donor to ubiquinone (Yano and Ohnishi, 2001). N2 iron–sulphur cluster is most likely located in the connection between the peripheral and the membrane arm. The magnetic interaction with the semiquinone radical, corresponding to a distance of about 10 Å (Magnitsky et al., 2002; Ohnishi and Salerno, 2005), suggests that the ubiquinone headgroup could somehow reach up into the peripheral arm as recently assumed by Brandt et al. (2003), who have hypothesized an amphipathic 'ramp' guiding ubiquinone into the catalytic site. Recently the arrangement of iron–sulphur clusters in the hydrophilic domain of Complex I from *Thermus thermophilus* has been determined by x-ray crystallography, showing a linear chain of all clusters except N1a and N7 (Hinchliffe and Sazanov, 2005).

Complex I is involved in the formation of the transmembrane proton gradient with a stoichiometry of 4 H^+/

Fig. 1. Cartoon of Complex I showing the electron pathway from FMN to the physiological acceptor CoQ and the reduction sites for the hydrophilic electron acceptor DCIP (2,4-dichloro-phenol indophenol).

$2e^-$. The limited knowledge about the mechanism of electron transfer of Complex I makes it difficult to predict the mechanism by which this respiratory chain complex uses redox energy to translocate protons across the inner mitochondrial membrane (cf. Section 3.2; for a review see Brandt, 1997).

Besides its well-known redox role in the electron transport chain, Complex I is considered one of the main sites of production of Reactive Oxygen Species (ROS): electron leaks at Complex I can release single electrons to oxygen and give rise to superoxide anion. The mechanism of superoxide production by Complex I is not clear, given the lack of knowledge of the exact sequence of the electron carriers and how electron transfer is coupled to proton translocation. The major sites of ROS production in the mitochondrial electron transport chain have been localized in Complex I and Complex III (Raha and Robinson, 2000); while the site of electron escape in Complex III has been identified in the so called centre "o", the direct oxygen reductant site in Complex I is not yet known with certainty (Andreyev et al., 2005).

Complex I is inhibited by more than 60 different families of compounds (Degli Esposti, 1998) starting from rotenone, the prototype of this series, to a number of synthetic insecticides/acaricides. These inhibitors were grouped into three classes based on their effects on the kinetic behaviour of the enzyme, having as prototypes piericidin A, rotenone, and capsaicin, respectively. Nevertheless kinetic studies did not allow different binding sites to be assigned for these three classes of inhibitors: it is commonly accepted that they share the same hydrophobic large pocket in the enzyme (Okun et al., 1999). These inhibitors have been widely exploited to understand the mechanism of electron transfer, proton translocation, and ROS formation by the enzyme.

The notion of Complex I as an individual enzyme stems out of its isolation as a discrete lipoprotein unit by detergent fractionation (Green and Tzagoloff, 1966). Recent structural and kinetic evidence, however, strongly suggests that complexes I and III form stable functional super-complexes (Schägger, 2001; Bianchi et al., 2004).

2.1.2. Complex II

Besides its functional role as succinate dehydrogenase in the Krebs cycle, the enzyme is involved in aerobic metabolism by the respiratory chain since it can couple the two-electron oxidation of succinate to fumarate with the electron transfer directly to the quinone pool; hence Complex II is more precisely termed succinate:quinone oxidoreductase (SQR) (Lancaster, 2002). There is no evidence that Complex II can act as a proton pump. It has been suggested that this is because the reaction catalyzed is not sufficiently exergonic to promote proton translocation (Schultz and Chan, 2001).

Various classification schemes have been proposed for Complex II based upon *in vivo* function, particularly the quinone substrate used by the enzyme, differences in *b*
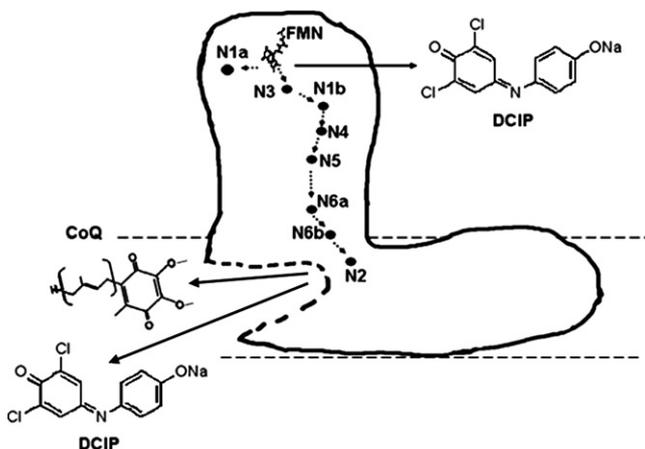
heme composition, and the number of membrane domain polypeptides (Lancaster, 2002). Mammalian Complex II is part of a class in which the enzymes are poised to reduce ubiquinone, contain a single *b* heme and are anchored to the inner mitochondrial membrane by two hydrophobic subunits, SdhC (14.2 kDa) and SdhD (12.8 kDa). While the membrane anchor domain shows low sequence identity (<20%) and varies in composition between organisms, the primary sequence of the soluble domain of Complex II is highly conserved (30–50% sequence identity) and consists of a flavoprotein subunit (SdhA, Fp, 64–79 kDa) containing covalently linked FAD and an iron–sulphur protein subunit (SdhB, Ip, 27–31 kDa), both located on the matrix side of the membrane. In the eukaryotic organisms, with only a few exceptions all the genes for Complex II are nuclear encoded (Scheffler, 1998; Figueroa et al., 2002; Burger et al., 1996).

The X-ray structure of SQR from *Escherichia coli* (Tornroth et al., 2002), analogous to the mitochondrial respiratory Complex II, has provided new insight into SQR's molecular design and mechanism, revealing the electron transport pathway through the enzyme. One feature of the Complex II structure is a linear electron transport chain that extends from the flavin and Fe–S redox cofactors in the extrinsic domain to the quinone and heme *b* cofactors in the membrane domain (Cecchini, 2003).

The interaction of quinones with Complex II is in an area located at the fringe of a hydrophobic pocket comprised of residues from three subunits (SdhB, SdhC and SdhD); structural data from *E. coli* has shed light on the amino acid residues that line the binding site proximal to a [3Fe–4S] cluster (Horsefield et al., 2006). Close inspection of the protein environment at the Q-site, by computational analysis using a protein–ligand docking program, confirmed that only two isoprene units of ubiquinone could be modelled in the native structure of the enzyme (Horsefield et al., 2006). To fully reduce the quinone in SQR, two protons and two electrons from the oxidation of succinate are needed. This would require two protons to be donated to ubiquinone by the protein environment of the Q-site followed by re-protonation of the site after catalytic turnover. In the native structure of SQR, Horsefield et al. (2006) had identified a proton uptake pathway suitable for such a purpose that crosses the membrane anchor arriving at the Q-site. The *E. coli* SQR Q-site is homologous to that of mammalian SQR based on an absolute conservation of amino acids in contact with ubiquinone, suggesting the same mechanism for electron transfer to ubiquinone, thus making it an excellent model system for mitochondrial Complex II research (Yankovskaya et al., 2003; Sun et al., 2005). This is of particular interest in humans, because mutations in Complex II result in various physiological disorders (Rustin et al., 2002).

2.1.3. Mitochondrial glycerol-3-phosphate dehydrogenase

FAD-linked mitochondrial glycerol-3-phosphate dehydrogenase (mtGPDH) is an enzyme of the inner mitochondrial membrane that shuttles reducing equivalents from the cytosol via the respiratory chain to molecular oxygen (Estabrook and Sacktor, 1958; Berry et al., 1973; Williamson et al., 1970). This metabolic shuttle was first discovered in insect flight muscle (Zebe and McShan, 1957; Estabrook and Sacktor, 1958) and in brown adipose tissue (Houstek et al., 1975) where the enzyme has highest activity. In pancreatic islet beta cells many studies support the significant participation of the shuttle in the events signalling the release of insulin in response to increased glucose (Malaisse, 1995). The mtGPDH is a very hydrophobic protein of the inner membrane; its catalytic centre is accessible from the outer surface of the inner membrane (Klingenberg, 1970; Donnellan et al., 1970).

Besides FAD, ascorbic acid stimulation (Jung and Wells, 1997) and the inhibition by di-iron metallo-enzymes inhibitors (Wells et al., 2001) suggest the presence of an iron–sulphur centre, that has however not been characterized. Its activity is regulated by acyl-CoA esters (Rauchova et al., 1993), free fatty acids (Rauchova and Drahota, 1984; Bukowiecki and Lindberg, 1974), and induced by hormones (Beleznai et al., 1989; Su and Lardy, 1991). The enzyme is calcium-dependent (Donnellan et al., 1970; Carafoli and Sacktor, 1972; Beleznai et al., 1990): being exposed to the outer surface of the inner membrane (Klingenberg, 1970; Donnellan et al., 1970) it is exposed to fluctuations in cytoplasmic calcium concentrations (Nichols and Denton, 1995); the binding site is part of the polypeptide chain of the enzyme, contrary to other calcium-sensitive mitochondrial dehydrogenases where calcium appears to bind separate subunits (Nichols and Denton, 1995). A specific activation by short-chain CoQ homologues (Rauchova et al., 1992) and by the CoQ analogue idebenone (unpublished results) was related to the release of the inhibitory effect of free fatty acids (Rauchova et al., 2003). This competition suggested that the inhibitory effect of free fatty acids is exerted by occupying the CoQ-reducing site of the enzyme, thus preventing transfer of reducing equivalents to the CoQ pool. The mechanism of CoQ reduction by the enzyme is not understood; the observation that CoQ homologues reduce superoxide formation by the enzyme in presence of ferricyanide (Drahota et al., 2002) suggests a very specific mechanism of interaction of CoQ with this enzyme, since these effects are not shared by either Complex I or Complex II.

Glycerol-3-phosphate dehydrogenase appears to interact directly with the CoQ pool thereby not forming a supramolecular aggregate with Complex III (Rauchova et al., 1997) (cf. Section 2.3).

2.1.4. ETF-ubiquinone oxidoreductase

The electron-transfer flavoprotein (ETF)-ubiquinone oxidoreductase (ETF-QO) is a globular protein located on the matrix surface of the inner mitochondrial membrane. The enzyme can accept reducing equivalents from a variety of dehydrogenases (Beckmann and Frerman, 1985), including those involved in fatty acid oxidation or

amino acid and choline catabolism, and is oxidized by the diffusible ubiquinone pool that also is accessed by other mitochondrial enzymes involved in electron transfer to and from CoQ (Simkovic and Frerman, 2004).

Crystal structures of the enzyme (Zhang et al., 2006) indicate that the molecule forms a single structural domain where three closely packed functional regions bind FAD, the 4Fe–4S cluster and ubiquinone. The ubiquinone molecule penetrates deep into its binding pocket, that consists mainly of hydrophobic residues, and the O1 carbonyl oxygen in the benzoquinone ring is hydrogen-bonded to the polypeptide chain. Only five units of the presumed 10 isoprenes in the flexible tail of CoQ could be seen in the structure of the ubiquinone-containing protein. According to Zhang et al. (2006), the binding mode of ubiquinone observed in the ETF dehydrogenase is different from those observed in most other CoQ-binding proteins whose binding motif shows semi-conserved sequences containing a Tyr/Trp or His that make direct hydrogen bonds to both carbonyl groups in the benzoquinone ring.

2.1.5. Dihydro-orotate dehydrogenase

Dihydroorotate dehydrogenase (DHODase) is an iron-containing 43-kDa flavoprotein (FMN) that catalyzes the oxidation of dihydroorotate to orotate, the fourth step in *de novo* pyrimidine biosynthesis (Evans and Guy, 2004).

Biochemical and microscopic studies (Hines et al., 1986; Miller, 1971) showed that the mammalian DHODase and that isolated from *Neurospora crassa* are integral membrane proteins localized in the inner mitochondrial membrane with the active site facing the inter-membrane space. The enzyme is functionally linked to the electron transport system of the respiratory chain since it uses ubiquinone as co-substrate electron acceptor (Jones, 1980; Miller, 1971), thus it classifies as a dihydroorotate:ubiquinone oxidoreductase. By contrast, the rat liver DHODase lacks flavin, contains iron and zinc as the two apparent redox-active cofactors and, like the cytosolic enzymes isolated form parasitic protozoa (Pascal et al., 1983; Pascal and Walsh, 1984), delivers electrons directly to molecular oxygen (Forman and Kennedy, 1977).

The N-terminus of the DHODase protein contains a bipartite signal that governs import and correct insertion into the mitochondrial membrane (Rawls et al., 2000). The high-resolution crystal structure of human DHODase (Liu et al., 2000), truncated of the bipartite sequence, shows a large domain containing the active site and a small domain that forms the opening of a tunnel leading to the bound FMN and that provides access to ubiquinone. Anti-proliferative agents related to brequinar and leflunomide (Jockel et al., 1998) share a common binding site in this tunnel blocking access of ubiquinone to the active site. Orotate is completely buried on the distal side of FMN so it is unlikely to enter via the same tunnel.

Analysis of the steady-state kinetic mechanism of highly purified bovine liver mitochondrial DHODase, as probed using deuterated substrates (Hines and Johnston, 1989b),

suggests that the enzyme may follow a rapid equilibrium random (two-site) hybrid ping-pong mechanism, typical of an enzyme that contains two non-overlapping and kinetically isolated substrate binding sites. According to the catalytic properties described by Hines and Johnston (1989a), it seems reasonable that FMN functions as the proximal electron acceptor, experiencing two-electron reduction concomitant with dihydroorotate oxidation. Reduced flavin would then become re-oxidized by passing electrons, perhaps one at a time, to the putative iron–sulphur cluster that, in turn, would be exposed to the quinone. *In situ*, the reduced ubiquinone would be expected to equilibrate with the membrane CoQ-pool and to be re-oxidized by the mitochondrial Complex III.

2.1.6. Alternative NADH dehydrogenases

Alternative NADH dehydrogenases (ND) designate a family of proteins located in the inner membrane of eukaryotic mitochondria, which catalyze oxidation of NAD(P)H from either the cytosol (external enzymes) or the mitochondrial matrix (internal enzymes) and enable quinone reduction. The greatest functional difference from Complex I is that their oxidoreductase activity is rotenone insensitive and is not coupled to proton pumping.

The number and specificity of alternative NADH dehydrogenases vary considerably when comparing different organisms: none was described in humans, while plants may have up to four proteins (two in each side of the membrane, but not yet conclusively identified) (Kerscher, 2000; Rasmusson et al., 1998), suggesting that they may have organism-specific roles.

Alternative dehydrogenases are also present in bacteria (Yagi et al., 1998) as well as in the mitochondria of fungi (Kerscher, 2000). In *N. crassa* mitochondria (Videira and Duarte, 2002), both internal and external rotenone-insensitive alternative NADH dehydrogenases have been proposed many years ago (Weiss et al., 1970). Later, an external calcium-sensitive NAD(P)H activity and higher activity of an internal enzyme in the early exponential phase of fungal growth were reported (Schwitzguebel and Palmer, 1982; Moller et al., 1982). In the yeast *S. cerevisiae*, which lacks Complex I, an internal and two external enzymes have been quite well characterized, respectively NDI1, NDE1 and NDE2 (De Vries et al., 1992; Overkamp et al., 2000). Another yeast, *Yarrowia lipolytica*, only contains one external enzyme in the inner mitochondrial membrane in addition to Complex I (Kerscher et al., 1999).

Alternative NADH dehydrogenases are encoded by a single nuclear gene and have a mature peptide molecular mass of 50–60 kDa. The only prosthetic group is FAD, by contrast with the FMN and multiple FeS centres of Complex I. Recent data showed that yeast NDI1 extracted by dodecyl-β-D-maltoside contains substoichiometric amounts of CoQ_8 and that the ratio of bound CoQ/NDI1 can increase to 1 after incorporation of exogenous quinone into the purified enzyme (Yamashita et al., 2007). This observation is consistent with previous reports

for *E. coli* NDH-2 extracted using cholate (Jaworowski et al., 1981). Clearly, the properties of bound CoQ in NDI1 are distinct from those of CoQ as substrate, suggesting that there are two binding sites in the enzyme (Yamashita et al., 2007). Because semiquinone has not been measured yet in NDI1, it is at present uncertain whether the boud CoQ of NDI1 participates in the electron transfer from FAD to CoQ pool. However, the importance of bound CoQ molecules was demonstrated in reconstitution experiments in which the presence of CoQ-bound NDI1 was shown to restore KCN- and antimycin-sensitive NADH oxidase activity of bovine submitochondrial particles in the presence of piericidin (Yamashita et al., 2007).

Gene cloning has established that NDA and NDB in potato mitochondria are markedly similar to the yeast NDI1, with sequence identity of about 30–40% (Rasmusson et al., 1999). Both NDA and NDB have NADH and FAD binding motifs, while neither has any indication of membrane spanning α-helices. Peptide uptake studies showed that the NDA precursor was readily imported into mitochondria, with removal of a short targeting peptide after transit. By contrast, the NDB precursor did not reach the mitochondrial matrix, despite an N-terminal targeting sequence. Instead, it only crossed the outer mitochondrial membrane. Both proteins became bound to the inner mitochondrial membrane, but their different targeting led to locations on opposite sides. Relative to the centre of the lipid bilayer, they would be almost mirror images of each other (Joseph-Horne et al., 2001), as shown in Fig. 2.

The cellular role and need for alternative NADH dehydrogenases is not clearly established. These enzymes and the proton-pumping Complex I have overlapping roles in oxidoreductase reactions and there is already evidence that alternative NADH dehydrogenases can complement Complex I defects in different situations. Disruption of Complex

Fig. 2. Organization of alternative internal and external NADH dehydrogenases. The internal and external NADH dehydrogenases of yeast (NDI1 and NDE1, respectively) are depicted as similar proteins with opposite topologies in the inner mitochondrial membrane. The experimental evidence relates to potato mitochondria, as discussed in the text. (Reprinted from Biochim. Biophys. Acta - Bioenergetics 1504, T. Joseph-Horne, D.W. Hollomon and P.M. Wood, Fungal respiration: a fusion of standard and alternative components, 179–195, ©(2001), with permission from Elsevier).

I genes in *Paracoccus denitrificans* was only possible after introduction into the organism of the NDH-2 gene of *E. coli* (Finel, 1996). Likewise, the segregation of Complex I mutants in *Y. lipolytica* required the previous targeting of its external single alternative NADH dehydrogenase to the matrix face of the inner mitochondrial membrane (Kerscher et al., 2001a). Moreover, the complementation of Complex I defects in mammalian cells with the NDI1 gene of *S. cerevisiae* is quite amazing and points to a possible strategy for gene therapy in human mitochondrial diseases (Yagi et al., 2001).

Since alternative NADH dehydrogenases do not pump protons, they may be useful to keep reducing equivalents at physiological levels and to avoid the active oxygen production associated with Complex I. Moreover, data on the characterization of the expression and activity regulation of these enzymes is emerging: alternative components respond to factors ranging from oxidative stress to the stage of fungal development. For instance, their direct involvement in oxidative stress in yeast (Davidson and Schiestl, 2001) or in development and light responses in plants (Svensson and Rasmusson, 2001) was described. Their variability among species is a sign that they accomplish specific requirements of the different organisms.

2.1.7. Complex III

The cytochrome bc_1 complex (ubiquinol-cytochrome c oxidoreductase, E.C. 1.10.2.2) from mitochondria of several species has been crystallized and its structure solved to atomic resolution (Iwata et al., 1998). The mechanism of the enzyme is generally well understood, although some questions remain outstanding.

In mitochondria, the bc_1 complex (Complex III) is located in the inner membrane where it is essential for cell respiration. The enzyme represents a confluence point for reducing equivalents from various dehydrogenases: it can catalyze the transfer of electrons from hydroxyquinones (ubiquinol, reduced CoQ) to a water-soluble c-type cytochrome and it can, concomitantly, link this redox reaction to translocation of protons across the membrane, converting the energy associated with the respiratory chain electron flow into an electrochemical proton gradient (Crofts et al., 2006).

All cytochrome bc_1 complexes contain three protein subunits with redox prosthetic groups, i.e. a di-heme cytochrome b containing a relatively high-potential b_H heme and a lower potential b_L heme, cytochrome c_1 and a Rieske iron–sulphur protein with a 2Fe-2S cluster (Berry et al., 2000). As many as seven or eight supernumerary subunits are also present in the mitochondrial enzymes. Possible functions for these non-redox subunits, that are not required for electron transfer and proton translocation activities of the enzyme, include structural stability and regulation of coordinated activity of the dimeric enzyme (Akiba et al., 1996), and docking sites for ternary complex formation with the dehydrogenase and oxidase complexes (Schafer et al., 2006).

The mitochondrial Complex III is a symmetrical, oligomeric dimer; it has been demonstrated that the iron–sulphur protein spans the dimer structure since it is anchored in one monomer while its peripheral domain is located in the other monomer, where it forms part of the ubiquinol oxidation site. Evidence exists that the dimer behaves as a functional monomer, on the basis of stoichiometry of inhibitor action on enzyme activity (Covian and Trumpower, 2006). Striking evidence exists that the peripheral domain of the Rieske protein moves back and forth between positions close to cytochrome b and cytochrome c_1 (Nett et al., 2000) facilitating electron transfer within the enzyme. Crystal structures established the location of the ubiquinol oxidation and ubiquinone reduction at topographically separated sites within each monomer and demonstrated the transmembrane disposition of the b hemes. These structural details provided a final confirmatory evidence of the proton-motive Q cycle mechanism of the enzyme, with protons being carried across the inner mitochondrial membrane while electrons from ubiquinol are transferred through the bc_1 complex (Osyczka et al., 2005).

2.1.8. Alternative quinol oxidases

Although Complex III is the only ubiquinol-oxidizing enzyme in mammalian mitochondria, most plants and some yeasts and fungi posses a cyanide- and antimycin-insensitive alternative oxidase (AOX) that catalyzes the aerobic oxidation of ubiquinol in addition to the cytochrome pathway (Krab, 1995). The enzyme is non-proton-motive and its activity does not contribute to the conservation of energy that can therefore be dissipated as heat (Moore et al., 1978; Meeuse, 1975). However, since the ubiquitous presence of AOX in plants including non-thermogenic species, a main hypothesis have been suggested for a more general physiological role of the enzyme as an overflow mechanism. It has been predicted that AOX allows Krebs-cycle turnover when the energy state of the cell is high and that it protects against oxidative stress (Juszczuk and Rychter, 2003).

Despite the difficulty of purification of the enzyme to homogeneity in a stable, active form (Zhang et al., 1996), recent models considered AOX as an approximately 32-kDa homo-dimeric interfacial protein peripherally associated with the matrix side of the inner mitochondrial membrane (Andersson and Nordlund, 1999; Siedow and Umbach, 2000). The structure of the active site of the oxidase comprises a non-heme di-iron centre that is reduced by two electrons delivered from ubiquinol (Berthold et al., 2000; Affourtit et al., 2002). Appreciable activity of the AOX can be observed only when the CoQ pool is in a reduced state; significant engagement of the alternative pathway is not apparent until pool reduction levels reach 40%. Siedow and Moore (Moore and Siedow, 1991; Siedow and Moore, 1993) proposed a detailed model to explain this kinetic characteristic. The model, based on CoQ pool behaviour, includes reaction of AOX with two quinol molecules to reduce a single oxygen and predicts the changing affinity for oxygen upon changes in CoQ-pool reduction. However, during titrations of the behaviour of the alternative pathway in response to CoQ-pool reduction, the maximum engagement of AOX appeared at levels of pool reduction less than 80–90%. This phenomenon was attributed to there being a fraction of the total ubiquinone pool that is not accessible to the alternative oxidase. This inaccessibility may be due to the existence of multiple quinone pools as a possible consequence of an heterogeneous distribution of the electron transfer complexes within the mitochondrial membrane (Ian et al., 1983; Rich et al., 1977).

Moreover, not only the amount of alternative oxidase (Juszczuk and Rychter, 2003), but also its kinetic characteristics vary with tissue conditions; a clear example is afforded by mitochondria isolated from young and mature spadices of *Arum maculatum* where the plot of AOX activity rate vs. Q-pool reduction changes from nonlinear to nearly linear as a function of tissue growth stage. Various regulatory phenomena that affect the activity of the alternative oxidase have been reported in the literature (Siedow and Moore, 1993).

2.2. Supra-molecular organization of the respiratory chain. Random distribution vs. super-complexes

The first proposal that CoQ functions as a mobile electron carrier was given by Green and Tzagoloff (1966) on the basis of the isolation of discrete lipoprotein complexes of the respiratory chain, of which the quinone was a substrate in excess concentration over the prosthetic groups in the complexes. The random collision model was proposed on the basis of direct investigation of the mobility of mitochondrial components by either electrophoretic relaxation or FRAP (fluorescence recovery after photobleaching), yielding lateral diffusion coefficients in the range between 10^{-9} and 10^{-10} cm^2/s for mitochondrial membrane complexes (Hackenbrock et al., 1986) and higher than 10^{-9} cm^2/s for CoQ (Hackenbrock et al., 1986; Lenaz and Fato, 1986). The long-range diffusion measured by FRAP (μm) could be slower than short-range diffusion (nm), as the result of the high density of proteins (Green and Tzagoloff, 1966). Accordingly, the diffusion coefficient of Complex III was strongly enhanced by phospholipid enrichment of the membrane (Hackenbrock et al., 1986; Sowers and Hackenbrock, 1985); significantly, the long-range diffusion coefficient of phospholipids, though also increased, was affected to much lesser extent.

The model was supported by the kinetic analysis of Kröger and Klingenberg (1973a); they showed that steady-state respiration in submitochondrial particles from beef heart could be modelled as a simple two-enzyme system, the first causing reduction of ubiquinone and the second causing oxidation of ubiquinol. If diffusion of the quinone and quinol species is much faster than the chemical reactions of CoQ reduction and oxidation, the quinone behaves kinetically as a homogeneous pool (cf. 2.3).

Differential scanning calorimetry, detecting the thermotropic behaviour of lipids removed from direct contact with proteins, showed that the majority of phospholipids in the inner mitochondrial membrane behave as a free bilayer (Hochli and Hackenbrock, 1979). Freeze-fracture electron microscopy showed that the intra-membrane particles are randomly distributed in the inner membrane (Sowers and Hackenbrock, 1981); although this can be taken as evidence for a random distribution of protein complexes in the lipid bilayer, it is not possible from microscopy alone to attain quantitative evaluations of the relative area of the membrane occupied by proteins and lipids.

The evidence for a random distribution of mitochondrial complexes, freely mobile in the inner membrane, was further supported by studies of rotational mobility (Dixit and Vanderkooi, 1984), showing that the rotational correlation time of cytochrome oxidase is the same whether Complex III and cytochrome c are present or not in the same reconstitution system (Kawato et al., 1981).

Additional evidence for a random distribution of electron transfer complexes stems from the fact that antibodies against Complex III and Complex IV aggregate these complexes separately (Hackenbrock and Hammon, 1975).

On the other hand, circumstantial evidence against a random distribution of respiratory complexes comes from isolation of Complex I – Complex III (Hatefi et al., 1962) and Complex II – Complex III units (Yu et al., 1974; Yu and Yu, 1980) having integrated enzyme activities, and also cytochrome b_6f-photosystem I units (Boardman, 1971) indicating that such units may be preferentially associated in the native membrane.

Because early reports of combined complexes used bile salts, which can lead to protein aggregations, and associations of complexes were not detected by antibodies within the membranes (Hackenbrock and Hammon, 1975), not many researchers paid attention to a potential supramolecular organization of the respiratory chain. Stable super-complexes of Complex III and IV isolated from *Paracoccus denitrificans* (Berry and Trumpower, 1985), thermophilic *Bacillus* PS3 (Sone et al., 1987) and thermoacidophilic archeon *Sulfolobus* (Iwasaki et al., 1995) seemed to be special to these bacteria.

More recently Cruciat et al. (2000) and Schägger (Schägger, 2001; Schägger and Pfeiffer, 2001) have produced new evidence of multi-complex units in yeast and mammalian mitochondria introducing a quantitative approach: a mild one-step separation protocol for the isolation of membrane protein complexes, namely Blue Native Polyacrylamide Gel Electrophoresis (BN-PAGE). In particular, BN-PAGE in digitonin-solubilized mitochondria of *Saccharomyces cerevisiae*, which possesses no Complex I, revealed two bands with apparent masses of ∼750 and 1000 kDa containing the subunits of complexes III and IV, as assigned after two dimensional SDS–PAGE followed by N-terminal protein sequencing (Schägger and Pfeiffer, 2000). The smaller super-complex (III_2IV_1) consisted of a Complex III dimer and a Complex IV monomer while the larger super-complex (III_2IV_2) represented a Complex III dimer associated with two Complex IV monomers. The minimal effect of increasing the digitonin/protein ratio for solubilization of mitochondria seemed to indicate a stable rather than a dynamic association of proteins.

Similar interactions of super-complexes were investigated in bovine heart mitochondria: Complex I–III interactions were apparent from the presence of a I_1III_2 super-complex that was found further assembled into two major super-complexes (respirasomes) comprising different copy numbers of Complex IV ($I_1III_2IV_2$ and $I_1III_2IV_4$). Only 14–16% of total Complex I was found in free form in the presence of digitonin (Schägger and Pfeiffer, 2001); so it seems likely that all Complex I is bound to Complex III in physiological conditions (i.e. in the absence of detergents). Knowing the accurate stoichiometry of oxidative phosphorylation complexes according to Schägger and Pfeiffer (2001), the average ratio I:II:III:IV:V is 1.1:1.3:3:6.7:3.5 therefore it is plausible that approximately one-third of total Complex III in bovine mitochondria is not bound to monomeric Complex I. The fraction of Complex IV in free form represents >85% of total cytochrome oxidase of mitochondria. Associations of Complex II with other complexes of the OXPHOS system could not be identified under the conditions of BN-PAGE so far.

BN-PAGE has become a popular experimental strategy for the structural analysis of the protein-complex composition of the respiratory chain in different systems. Based on this procedure, the existence of respirasome-like super-complexes was also reported for bacteria (Stroh et al., 2004), fungi (Krause et al., 2004a) and higher plant mitochondria (Eubel et al., 2004b; Krause et al., 2004b) as well as for human mitochondria (Schägger et al., 2004).

The I–III super-complex proved to be especially stable. Depending on the plant investigated, 50–90% of Complex I forms part of the I_1III_2 super-complex in *Arabidopsis* (Dudkina et al., 2005), potato (Eubel et al., 2004a), bean and barley (Eubel et al., 2003) upon solubilisation of isolated mitochondria by nonionic detergents; whereas Complex IV-containing super-complexes are of low abundance (bound Complex IV is <10%) and Complex II clearly is not associated, even in plants.

The first chromatographic isolation of a complete respirasome ($I_1III_4IV_4$) from digitonin-solubilised membranes of *P. denitrificans* indicated that Complex I is stabilized by assembly into the NADH oxidase super-complex since attempts to isolate Complex I from mutant strains lacking complexes III or IV led to the complete dissociation of Complex I under the conditions of BN-PAGE. Reduced stability of Complex I in those mutant strains was also apparent from an almost complete loss of NADH-ubiquinone oxidoreductase activity when the same protocol as for parental strain was applied (Stroh et al., 2004).

Analysis of the state of super-complexes in human patients with an isolated deficiency of single complexes (Schägger et al., 2004) and in cultured cell models harbouring cytochrome b mutations (Acin-Perez et al., 2004;

D'Aurelio et al., 2006) also provided evidence that the formation of respirasomes is essential for the assembly/stability of Complex I. Genetic alterations leading to a loss of Complex III prevented respirasome formation and led to secondary loss of Complex I, therefore primary Complex III assembly deficiencies presented as Complex III/I defects. Conversely, Complex III stability was not influenced by the absence of Complex I. D'Aurelio et al. (2006) studied mtDNA complementation in human cells by fusing two cell lines, one containing a homoplasmic mutation in a subunit of respiratory chain Complex IV, COX I, and the other a distinct homoplasmic mutation in a subunit of Complex III, cytochrome *b*. Upon cell fusion, respiration recovered in hybrid cells, indicating that mitochondria fuse and exchange genetic and protein materials. The recovery of mitochondrial respiration correlated with the presence of supra-molecular structures (supercomplexes) containing complexes I, III and IV; critical amounts of complexes III or IV are therefore required in order for super-complexes to form and provide mitochondrial functional complementation. From these findings, super-complex assembly emerged as a necessary step for respiration, its defect setting the threshold for respiratory impairment in mtDNA mutant cells.

Despite mounting evidence based on electrophoretic results showing that respiratory protein complexes specifically interact forming super-molecular structures, characterization of the super-complexes by biochemical functional analysis is still poor. Kinetic testing using metabolic flux control analysis is a powerful source of information on the supra-molecular organization of enzyme complexes (Kacser and Burns, 1979; Moreno-Sanchez et al., 1999). (cf. Section 2.3).

2.3. Kinetic consequences of the supra-molecular organization of the respiratory chain: pool function vs. channeling

2.3.1. "Pool" behavior of CoQ

The first proposal that CoQ functions as a mobile electron carrier was given by Green and Tzagoloff (1966) on the basis of the isolation of discrete lipoprotein complexes of the respiratory chain, of which the quinone was a substrate in excess concentration over the prosthetic groups in the complexes, and was subsequently supported by the kinetic analysis of Kröger and Klingenberg (1973a); they showed that steady-state respiration in submitochondrial particles from beef heart could be modeled as a simple two-enzyme system, the first causing reduction of ubiquinone and the second causing oxidation of ubiquinol. If diffusion of the quinone and quinol species is much faster than the chemical reactions of CoQ reduction and oxidation, the quinone behaves kinetically as a homogeneous pool. According to this assumption, during steady-state electron transfer, the overall flux observed (V_{obs}) will be determined by the redox state of the quinone and propor-

tional to either reduced Q (Q_{red}) or oxidized Q (Q_{ox}) concentration:

$$V_{obs} = V_{ox}(Q_{red}/Q_t) = V_{red}(Q_{ox}/Q_t) \qquad (1)$$

where V_{ox} is the rate of ubiquinol ($CoQH_2$) oxidation, V_{red} is the rate of CoQ reduction and Q_t is total CoQ concentration (reduced plus oxidized). Manipulation of Eq. (1) leads to the *pool equation*

$$V_{obs} = (V_{red} \cdot V_{ox})/(V_{red} + V_{ox}) \qquad (2)$$

The hyperbolic relation of electron flux on the rate of either CoQ reduction (V_{red}) or $CoQH_2$ oxidation (V_{ox}) was confirmed in a variety of systems (Gutman, 1985; Ragan and Cottingham, 1985; Rich, 1984). The concept of a mobile, laterally diffusing pool of CoQ molecules linking dehydrogenases and bc_1 complexes is the natural consequence of these kinetic observations. Further evidence was provided by the characteristic effect of changing V_{red} or V_{ox} on inhibitor titration curves (Kröger and Klingenberg, 1973b).

According to this concept, pool behavior is characterized by a convex hyperbolic relationship between the integrated oxidation rate and the inhibitor concentration, whereas a linear relationship is expected by a stoichiometric association between the two enzymes.

A large body of experimental evidence concerning succinate oxidation has validated the pool equation in a variety of mitochondrial systems, whereas fewer data are available for NADH oxidation (Kröger and Klingenberg, 1973a; Fato et al., 1996; Gutman, 1985).

It might be argued that CoQ-pool behaviour was shown only in reconstituted systems (Norling et al., 1974; Zhu et al., 1982; Lenaz et al., 1989; Estornell et al., 1992) or in mitochondria where the relations of the inner membrane complexes were altered by swelling (Schneider et al., 1982), freeze-thawing cycles (Parenti Castelli et al., 1987), or sonication (Kröger and Klingenberg, 1973a), whereas in intact mitochondria inner-outer membrane contacts and the quasi-solid organization of the matrix (Srere, 1982) may keep the integral proteins in a clustered immobilized arrangement. A study dealing with presence of diffusible intermediates in the respiratory chain in intact phosphorylating mitochondria was accomplished by Stoner (1984) who showed that in intact coupled mitochondria in state 3 (i.e. synthesizing ATP), inhibition of Complex III with myxothiazol makes succinate oxidase less sensitive to the Complex II inhibitor 3′-hexylcarboxin, in accordance with the existence of a freely diffusible intermediate between the two steps (Baum, 1977). Unfortunately in his study Stoner did not perform similar titrations for search of a diffusible intermediate between complexes I and III.

In conclusion we may state with some certainty that, in beef heart mitochondria, succinate oxidation exhibits pool behavior, indicating the presence of CoQ as a diffusible intermediate between Complex II and Complex III; on the other hand, the same statement for NADH oxidation is supported by less clear-cut evidence.

If the CoQ concentration is not saturating for the activity of the reducing and oxidizing enzymes, the Eq. (1) is modified (Ragan and Cottingham, 1985) by feeding it in the Michaelis–Menten equation for enzyme kinetics, taking into account total CoQ concentration $[Q_t]$, the individual V_{max} of the dehydrogenase and Complex III and their dissociation constants for CoQ. V_{obs} is hyperbolically related to $[Q_t]$ and maximal turnovers of electron transfer are attained only at $[Q_t]$ saturating both V_{red} and V_{ox} (Lenaz and Fato, 1986).

$$V_{obs} = \frac{[(V_{mr} \cdot V_{mo})/(V_{mr} + V_{mo})] \cdot Q_t}{[(V_{mr} \cdot K_{so} + V_{mo} \cdot K_{sr})/(V_{mr} + V_{mo})] + Q_t} \quad (3)$$

where V_{mr} and V_{mo} are the maximal velocities of CoQ reduction and ubiquinol oxidation, respectively, and K_{sr} and K_{so} are the dissociation constants for the dehydrogenase and for Complex III, respectively. An additional complication in the saturation kinetics of CoQ may be in the fact that multiple quinone-binding sites may exist in the competent enzymes (e.g. site O or P and site I or N in Complex III, Trumpower (1981)) and that oxidized and reduced forms of CoQ may bind to the same binding sites with different affinities and mutually compete with one another.

The factor in square parenthesis at the denominator of Eq. (3) is the apparent K_m for Q_t of the integrated activity (NADH oxidation or succinate oxidation). Direct titrations of CoQ-depleted mitochondria reconstituted with different CoQ supplements yielded a "K_m" of NADH oxidation for Q_t in the range of 2–5 nmol/mg mitochondrial protein (Estornell et al., 1992), corresponding to a Q_t concentration of 4-10 mM in the lipid bilayer. The "K_m" in the composite system is a poised function of V_{max} and dissociation constants for CoQ of the complexes involved; this "K_m" can be therefore varying with rate changes of the complexes linked by the CoQ-pool, but is anyway an important parameter, in that it is operationally described as the Q_t concentration yielding half-maximal velocity of integrated electron transfer V_{obs} (Lenaz et al., 1997). Analysis of the literature shows that the physiological CoQ content of several types of mitochondria (Battino et al., 1990) is in the range of the K_m for NADH oxidation, and therefore not saturating for this activity. In contrast to NADH oxidation, the "K_m" for succinate oxidation for Q_t was found one order of magnitude lower (Estornell et al., 1992), although Norling et al. (1974) had found similar values for the two systems.

The relation between electron transfer rate and CoQ concentration was seen for NADH oxidation in reconstituted systems and in phospholipid-enriched mitochondria (Schneider et al., 1982; Parenti Castelli et al., 1987); in spite of the calorimetric and spin label evidence of Yu (Gwak et al., 1986), it seems that succinate oxidation obeys pool behavior, as it is dependent on concentration of the CoQ-pool (Estornell et al., 1992; Schneider et al., 1982).

The substrate-like nature of CoQ is also shown by the fact that it exhibits saturation kinetics, not only when a short homologue is used as a substrate for an individual enzyme, but also when the natural CoQ_{10} is titrated in integrated respiration (i.e. NADH- and succinate-cytochrome c reductase) (Lenaz and Fato, 1986; Ragan and Cottingham, 1985; Estornell et al., 1992). A puzzling observation is that the K_m for CoQ_{10} of NADH cytochrome c reductase is much higher than that of succinate cytochrome c reductase (Estornell et al., 1992): the latter is of the same order of magnitude of the concentration of respiratory enzymes, a possible suggestion in favor of a stoichiometric association of Complex II with Complex III, that is however not experimentally found in neither the BN-PAGE investigations (Schägger and Pfeiffer, 2001) nor in metabolic flux control analysis (Bianchi et al., 2004), but was however suggested by biophysical investigations (Gwak et al., 1986).

2.3.2. Deviations from pool behavior

Despite the wide acceptance of the CoQ pool as the mechanism for integrated electron transfer from dehydrogenases to cytochromes, deviations from pool behavior have also been described, raising doubts on the *universal* validity of the hypothesis (Ragan and Cottingham, 1985). Important deviations from pool behavior were reported by Gutman (1985) in beef heart submitochondrial particles oxidizing both NADH and succinate at the same time: since the predicted rates were smaller than the calculated rates, it was suggested that the two systems do not interact as closely as expected for a single homogeneous CoQ pool.

Other deviations from pool behavior occur at high membrane viscosity (Heron et al., 1979) and at low ubiquinone concentration (Zhu et al., 1982), when the diffusion of the few quinone molecules over large distances may be hampered and one dehydrogenase may reduce only one or few Complex III molecules within the distance scanned by diffusion, so that the system may approach solid-state behavior.

In *S. cerevisiae* mitochondria under physiological conditions, it was shown that neither ubiquinone nor cytochrome c exhibits pool behavior, as determined from inhibitor titration experiments, implying that the respiratory chain in yeast is one functional and physical unit, all respiratory complexes having a control coefficient of one on respiration (Boumans et al., 1998). On the other hand, addition of high phosphate or trichloroacetate, acting as chaotropic agents, restores pool behavior for both electron carriers. The authors concluded that the respiratory chain of yeast is organized as a supra-molecular unit, but ascribed their findings to a special feature of the respiratory chain in yeast at difference with higher eukaryotes where pool behavior is normally found.

2.3.3. Flux control analysis of the respiratory complexes

Kinetic testing using metabolic flux control analysis is a powerful source of information on the supra-molecular organization of enzyme complexes (Kacser and Burns, 1979; Moreno-Sanchez et al., 1999). *Metabolic control analysis* predicts that if a metabolic pathway is composed of distinct enzymes freely diffusible in a dynamic organiza-

tion, the extent to which each enzyme is rate-controlling may be different and the sum of all the flux control coefficients for the different enzymes should be equal to unity.

The *flux control coefficient* (C_i) of a step in a metabolic pathway is defined as the fractional change in the global flux through the pathway induced by a fractional change in the enzyme under consideration and it can be expressed in mathematical terms (Kacser and Burns, 1979) as the ratio between the change over the metabolic flux rate $(dJ/dI)_{I=0}$ and the corresponding infinitesimally small change of enzyme activity $(dv_i/dI)_{I=0}$ induced by a specific inhibitor.

On the other hand, in a super-complex, the metabolic pathway would behave as a single enzyme unit, and inhibition of any one of the enzyme components would elicit the same flux control. In particular, in a system in which the respiratory chain is totally dissociated from other components of the OXPHOS apparatus (i.e. ATP synthase, membrane potential, and carriers), such as open non-phosphorylating submitochondrial particles, the existence of a super-complex would elicit a flux control coefficient near unity at any of the respiratory complexes, and the sum of all coefficients would be above 1 (Kholodenko and Westerhoff, 1993).

The use of flux control coefficients for the assessment of pool behavior, although based on different theoretical grounds, has a practical exploitation very similar to that of inhibitor titrations: in fact the presence of a lag in the inhibition of the integrated activity, as an indication that the inhibited step is not rate-limiting, should be common to both types of analysis.

The problem was addressed in mammalian and in plant mitochondria. The flux control coefficients of the respiratory complexes (I, II, III, and IV) were investigated using bovine heart mitochondria and submitochondrial particles devoid of substrate permeability barriers (Bianchi et al., 2004). Both Complex I and III were found to be highly rate-controlling over NADH oxidation, a strong kinetic evidence suggesting the existence of functionally relevant association between the two complexes. On the contrary, Complex IV appears to be randomly distributed, although it is possible that if any stable interaction with Complex IV exists in mammalian mitochondria, it escaped detection most likely due to a pronounced abundance of molecules in non-assembled form. Moreover, Complex II is fully rate-limiting for succinate oxidation, clearly indicating the absence of substrate channeling toward complexes III and IV (Bianchi et al., 2004). The major results are shown in Table 1.

In permeabilized mitochondria from freshly harvested potato tubers, where no activity of the so called alternative oxidase, AOX, is present at the level of ubiquinone (Affourtit et al., 2001), inhibitor titration experiments on the rotenone-sensitive and rotenone-insensitive respiration indicate that Complex III and IV are involved in the formation of a super-complex assembly comprising Complex I whereas the alternative dehydrogenases, as well as the

Table 1
Flux control coefficients (C_i) in bovine heart mitochondria respiring on NADH or succinate

Control exerted by	NADH oxidase	Succinate oxidase
Complex I	1.06	n.a.
Complex II	n.a.	0.88
Complex III	0.90	0.34
Complex IV	0.26	0.20

Mitochondria were uncoupled particles devoid of substrate barriers by multiple freezing and thawing as indicated in Bianchi et al. (2004).
n.a., not applicable.

molecules of Complex II, are considered to be independent structures within the inner mitochondrial membrane (unpublished observations from our laboratory).

2.3.4. Functional relevance of respiratory super-complexes and role of CoQ pool

Although, from a structural point of view, little doubt exists that respiratory chain complexes are organized, at least in part, as supra-molecular aggregates, their functional significance is still obscure: the obvious consequence of such organization in providing contact surfaces for substrate (CoQ and cyt. *c*) channeling (Bianchi et al., 2004) must be reconciled with experimental evidence of a pool function of CoQ and cyt. *c* and of diffusional interactions at the basis of inter-complex electron transfer (Kröger and Klingenberg, 1973a,b; Gutman and Silman, 1972; Gutman, 1985; Zhu et al., 1982; Fato et al., 1996).

The problem should be restricted to NADH oxidation, since, in the system supporting succinate oxidation, Complex II does not physically interact with Complex III in a super-complex, as amply discussed above.

The physiological implications of the interaction between Complex I and Complex III are not yet fully understood. It was speculated that they include enhancing of electron flow by direct channeling of ubiquinone, but the matter should be examined in better detail.

One remaining question concerns the compatibility/incompatibility of the stoichiometric channeling of CoQ between Complex I and Complex III (Ozawa et al., 1987; Schägger and Pfeiffer, 2000, 2001) and the existence of pool behavior by the bulk of CoQ molecules (Capaldi, 1982; Fato et al., 1996; Genova et al., 1995; Battino et al., 1990) free in the bilayer.

Actually, in presence of preferential associations, the pool equation would be still validated if the rate of association/dissociation of the complexes were *faster* than the rate of electron transfer between complexes and CoQ molecules in the pool. An alternative possibility would be that, within a super-complex, CoQ reduced by one enzyme has to dissociate in the pool in order to meet any other super-complex, including the same one in a different site, in order to be oxidized (Fig. 3).

On the other hand, the bound inter-complex quinone that allows electron flow directly from Complex I to Complex III may well be in dissociation equilibrium with the
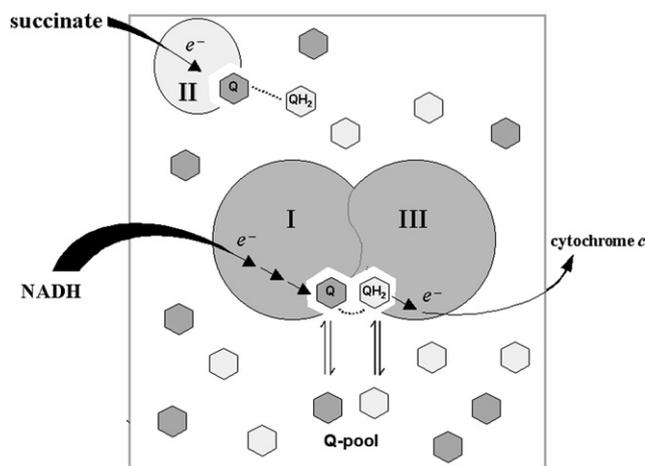
Fig. 3. Possible mechanism for the dissociation equilibrium of the bound inter-complex quinone with the CoQ pool. Kinetic evidence for intermediate channelling in the I–III super-complex requires the dissociation rate constants of ubiquinone and ubiquinol to be considerably slower than the rates of electron transfer via the same quinone molecules bound to the super-complex. Quinone interactions with Complex II are assumed to follow pool behaviour.

CoQ pool, so that its amount, at steady state, would be dictated by the size of the pool: this equilibrium would explain the saturation kinetics for total ubiquinone exhibited by the integrated activity of Complex I and Complex III (Estornell et al., 1992) and the decrease of respiratory activities in mitochondria fused with phospholipids with subsequent dilution of the CoQ pool (Schneider et al., 1982). To be in agreement with the experimental observation obtained by metabolic flux analysis, this proposition must however require that the dissociation of bound CoQ be considerably slower than its inter-complex electron transfer activity. In order to explain the high apparent K_m found for CoQ in NADH oxidase activity (Estornell et al., 1992) also the rate constant for CoQ binding to Complex I must be slow (Genova et al., 2005).

Conversely, Complex II kinetically follows pool behavior in reconstitution experiments (Estornell et al., 1992) and in the double inhibitor titration experiments (Stoner, 1984), in complete accordance with the lack of super-complexes found by both BN-PAGE (Schägger and Pfeiffer, 2001) and flux control analysis (Bianchi et al., 2004), but the isolation of discrete units having succinate cytochrome c reductase activity (Hatefi, 1978; Yu et al., 1974) and the biophysical studies described in (Gwak et al., 1986) appear in strong contrast with this concept. The only possible explanation so far is in the existence of very loose but specific contacts between Complex II and Complex III, not giving rise to any kind of channeling even in phosphorylating mitochondria (Stoner, 1984); these contacts are lost even in frozen mitochondria and in submitochondrial particles (SMP), as appears by BN-PAGE (Schägger and Pfeiffer, 2001), but are strongly non-physiologically enhanced by some treatments used for mitochondrial respiratory chain fractionation.

Of course the functional relevance of super-complex organization in electron transfer may vary with the physiological conditions in the intact cells: clearly under physiological conditions since the respiration rate is under control of energy consuming processes (respiratory control). Under prevalent State 4 conditions (high ATP/ADP ratio), the electrochemical proton gradient is largely the rate-limiting step, so that operation of the respiratory chain as tunneling or diffusion may be of minor importance for the flux control. On the other hand, the situation may be dramatically different under conditions of high energy demand (State 3) or under uncoupling conditions; the latter may be more important in vivo than originally thought: it has been suggested that muscle and liver mitochondria may dissipate 30–50% of the electrochemical potential as heat (Rolfe et al., 1999) through action of the uncoupling proteins (Ricquier and Bouillaud, 2000).

Recently, Piccoli et al. (2006) evaluated the impact of the mitochondrial trans-membrane potential ($\Delta\mu_{H+}$) on the flux control exerted by cytochrome c oxidase on the respiratory activity in intact cells. The results indicate that, under conditions mimicking the mitochondrial state 4 respiration, the control strength of the oxidase is decreased in respect to endogenous state 3 respiration. The authors suggest that such a change in control strength might be featured in terms of equilibrium between different organizational structures of the enzymatic complexes constituting the mitochondrial OXPHOS (Piccoli et al., 2006). Although the driving forces leading to the assembly/disassembly of the super-complexes have been not yet defined, it is not unconceivable that, given the membrane-integrated nature of the single complexes, electrostatic/hydrophobic interactions may enter into play in response to $\Delta\mu_{H+}$.

Nevertheless, a great deal of data in the literature (e.g. Gupte et al., 1984; Fato et al., 1986; Hackenbrock et al., 1986; Rajarathnam et al., 1989) indicate that most certainly a mobile pool of CoQ exists in the inner mitochondrial membrane, and that this pool is in equilibrium with protein-bound CoQ, as discussed above. A major function of the CoQ pool, therefore, must be to drive binding into sites formed at the border between adjacent complexes I and III in order to assure correct channeling of electrons from one to the other complex. Since the dissociation constant of CoQ from the super-complex must be high, as inferred from the apparent K_m of the integrated activity and previously discussed, any decrease of CoQ concentration in the pool would decrease the amount of bound CoQ and therefore induce a fall of electron transfer. By this way, free CoQ behaves as a reservoir for binding to the I–III super-complex; in addition, free CoQ may be a reservoir for other functions believed to require CoQ binding to specific proteins, such as uncoupling proteins (Echtay et al., 2000) and the permeability transition pore (Armstrong et al., 2003; Walter et al., 2002), and it also represents the main antioxidant species in the inner mitochondrial membrane (Ernster and Dallner, 1995).

Furthermore, other activities such as glycerol-3-phosphate dehydrogenase, ETF dehydrogenase, dihydroorotate dehydrogenase, that are likely to be in minor amounts and strongly rate-limiting in integrated electron transfer, are probably dictated by interaction through the CoQ pool. The only direct study addressed to this problem (Rauchova et al., 1992) demonstrated that in brown fat mitochondria the inhibition curve of glycerol phosphate cytochrome *c* reductase is sigmoidal in the presence of myxothiazol and antimycin, suggesting the presence of a homogeneous CoQ pool between glycerol phosphate dehydrogenase and Complex III. Also reverse electron transfer from succinate to NAD^+, involving sequential interaction of complexes II and I by means of CoQ, must take place by collisional interactions in the CoQ pool, since no aggregation exists between complexes I and II (Gutman, 1985).

This observation poses a particularly puzzling question: if Complex I is completely or almost completely associated with Complex III, and the interaction of CoQ in the pool with the quinone-binding site in common between the two enzymes is necessarily slow (see above), then how can $CoQH_2$ reduced by Complex II interact from the pool with the CoQ site in Complex I at a rate compatible with the steady state kinetics of reverse electron transfer? The intriguing idea that Complex I may possess two different quinone-binding sites for direct and for reverse electron transfer respectively is compatible with the proposal by Grivennikova et al. (2003) that two different routes exist for forward and reverse electron transfer within the complex. These two sites might become alternatively accessible depending on the magnitude of the membrane potential. Alternatively one should postulate that the association rate constant of *reduced* CoQ from the pool to Complex I in the super-complex should be sufficiently fast to be compatible with the turnover of reverse electron transfer. It must be noted that the ATP-driven reverse electron transfer from succinate to NAD^+ occurs in the presence of a high mitochondrial trans-membrane proton-motive force that, according to (Piccoli et al., 2006), might be the physiological signal and, at the same time, the trigger causing the structural reorganization of the enzymatic complexes of the mitochondrial OXPHOS system. The model hypothesis depicted by Piccoli et al. from data on cytochrome oxidase (Piccoli et al., 2006) might be extended to other enzymes of the respiratory chain, suggesting that also the I–III supercomplex would dissociate its constituting complexes under high $\Delta\mu_{H+}$ condition, and this would no longer limit the access to the CoQ binding site in Complex I.

3. Intra-complex electron transfer mediated by CoQ

3.1. Nature of the CoQ-binding sites in mitochondrial complexes

Several studies have suggested that a large amount of CoQ molecules in the mitochondrial membrane (up to 32% of the total content) can exist as a separate and distinct pool, not freely diffusable, which is associated with membrane proteins that possess selective affinity for CoQ (Lass and Sohal, 1999; Jorgensen et al., 1985; Ragan and Cottingham, 1985). The high resolution crystal structures of a number of quinone-reactive membrane proteins have revealed their quinone-binding sites (Q-sites) and facilitated their analysis (Berry et al., 1999; Horsefield et al., 2006; Breton, 2004). These structures and contributions from biochemical, mutagenic, inhibitor binding, EPR and IR studies (Ritter et al., 2003; Oyedotun and Lemire, 2001; Muller et al., 2003) have highlighted significant variation and no clear relationship between architectures and mechanisms of quinone binding, apart from that in the homologous family of "type II" photosynthetic reaction centres (Barber et al., 1999). It is also worth noting that significant variability is seen for quinone binding in the same protein from different species and that different redox states of quinones may adopt different conformations in the binding pockets.

Currently no common architecture or universal catalytic mechanism can be applied to Q-sites in general and several distinct types of Q-sites associated with respiratory electron transfer complexes can be specified although they can exhibit some very general analogous features. The quinone/quinol head group binds into the hydrophobic site primarily by interaction of appropriately placed hydrogen bonds with the carbonyl/hydroxyl moieties, and with the ring flanked by aromatic and aliphatic residues. Further interactions with the quinone side-chain occur to give steric limitations to possible antagonists. The presence of amino acid networks that might provide a proton-conducting hydrophilic channel through the protein radically changes the properties of the bound quinone molecule that, otherwise, can cycle only between the oxidised and semiquinone forms without being protonated to quinol.

A weak sequence motif associated with the presence of a Q-site has been proposed for the Q(A) and Q(B) sites of bacterial reaction centres (Fig. 4); α-helical regions of both L and M subunits in *Rhodobacter spheroides* contain a fully conserved hydrogen-bonding histidine residue (His) forming a triad of close contact residues with the fourth amino acid upstream and the third downstream of the His (Fisher and Rich, 2000). In its most general form, the triad is:

$$\text{aliphatic} - (X)_3 \text{-} H \text{-} (X)_{2/3} - (L/T/S)$$
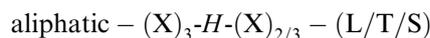
Sequences strongly resembling the histidine-containing triad can be identified in the Q(i) site of mitochondrial Complex III (Fisher and Rich, 2000), whereas no obvious similarities of regions of the Q(o) site, that is responsible for oxidation of quinol by Complex III, with those sites described above was noticed, and it seems likely that this site is unrelated in sequence or structure.

Analysis of sequence databases also indicated that a histidine-containing element, reminiscent of the triad sequence which forms one part of the Q(i) site in Complex III, may be present in the ND4 and ND5 subunits of Complex I and, possibly, in the mitochondrial alternative quinol
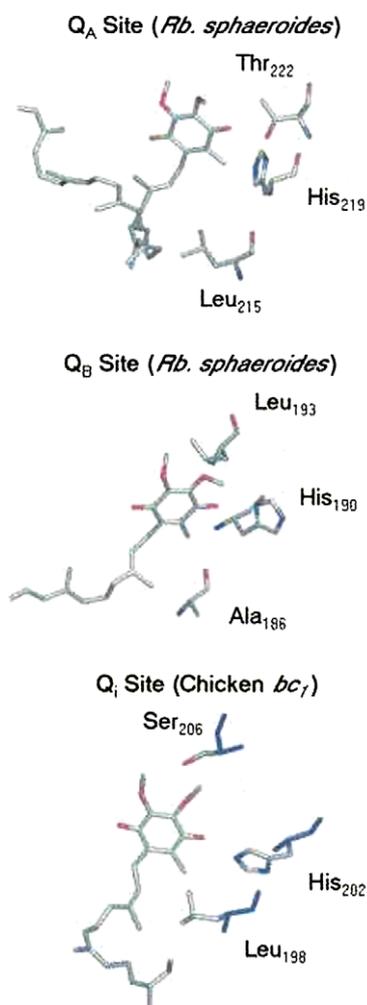
Fig. 4. Comparison of the structures of the quinone headgroup in relation to the amino acid triads of close contact residues in the bacterial QA and QB sites and in the Qi site of avian bc_1 complex. (Reprinted from J Mol Biol. 296, N. Fisher, P.R. Rich, A motif for quinone binding sites in respiratory and photosynthetic systems, 1153–1162, Copyright (2000), with permission from Elsevier).

oxidase (Fisher and Rich, 2000). Hydropathy plot prediction of membrane-spanning helices suggests that such well-conserved histidine residues in Complex I subunits are located close to the negative aqueous phase interface with which a quinone reduction site is expected to be in contact (Degli Esposti et al., 1994; Prieur et al., 2001). It has been suggested by Okun et al. (1999) that the ND4/ND5 pair might harbour two separate Q sites as a tightly bound QA-like site and an exchangeable QB-like site. However, the number of binding sites for ubiquinone in NADH-Q oxidoreductase is an unsolved question subject to intense controversy. Most suggestions were derived from studies involving labeled inhibitor analogues (Gong et al., 2003; Nakamaru-Ogiso et al., 2003) and up to three sites have been proposed in the literature (Vinogradov, 1993; Brandt, 1997).

In Complex II, several amino acid residues, identified by biochemical analysis as critical to the function of the enzyme, have a clear role in the mechanism of quinone binding and reduction, but comparison with other respiratory complexes indicates that the structural and inhibition patterns observed for the Q-binding site(s) in Complex II are different from those in Complex I and III (Horsefield et al., 2006; Tan et al., 1993).

At the moment, the question if tightly bound, not exchangeable CoQ molecules, similar to Q(A) in photosynthetic reaction centres exist in mitochondrial complexes is still an open question.

3.2. Mechanism of electron transfer in Complex I

The role of CoQ in the mechanism of electron transfer of respiratory complexes has been the subject of extensive research. The mechanism of Mitchell's Q-cycle in Complex III with a bifurcation of electrons from ubiquinol into two paths at high and low redox potential respectively is now well consolidated and we refer to excellent reviews on the topic (Mitchell, 1975; Cramer and Zhang, 2006; Crofts, 2004; Osyczka et al., 2005; Rich, 2004; Hunte et al., 2003; Brandt, 1996). On the other hand the mechanism by which electrons are fed from Complex I to CoQ is still object of debate; here we give a brief account of the state-of-the art.

The primary acceptor of electrons from NADH is FMN bound to the 51 kDa subunit (Fecke et al., 1994); since iron–sulphur cluster N1a has a very negative potential and is situated too far from the other iron–sulphur clusters (Hinchliffe and Sazanov, 2005), it is not considered to reside in the main pathway of electrons (Videira and Duarte, 2002). Thus electrons would flow from FMN to N3 in the same 51-kDa subunit, and to N4 and N5 in the 75-kDa subunit (Ohnishi, 1998; Yagi et al., 1998; Yano et al., 1995), and then to N6a and N6b in the TYKY subunit (Friedrich et al., 2000) and to N2 in PSST subunit (Sousa et al., 1999; Friedrich, 1998) but shared with the 49-kDa subunit (Kashani-Poor et al., 2001). N2 is the direct electron donor to bound ubiquinone (Ohnishi, 1998) and probably this step is linked to proton translocation (Yano and Ohnishi, 2001), although the mechanism is still debated (Degli Esposti, 1998; Brandt, 1997, 2006; Friedrich, 2001; Dutton et al., 1998; Vinogradov, 2001; Hirst, 2005; Ohnishi and Salerno, 2005; Yagi and Matsuno-Yagi, 2003). A recent view favours a conformational mechanism (Brandt et al., 2003; Brandt, 2006), since all redox groups in the enzyme appear to be located in the hydrophilic arm or at least at the interface with the hydrophobic arm. Moreover, the redox-Bohr effect conferred by a histidine in the 49-kDa subunit of the complex from Y. lipolytica is not required for proton translocation (Zwicker et al., 2006), suggesting that the reduction of CoQ at the interface between the 49-kDa and PSST subunits triggers long-range conformational changes transmitted to subunits of the membrane arm acting as actual proton pumping devices.

The mechanism of CoQ reduction is particularly intriguing, since more than one bound quinone species has been

assigned to the enzyme; three ubisemiquinone signals are detectable in the enzyme (Ohnishi et al., 1998; Ohnishi, 1998).

It is however unlikely that a strongly bound non-exchangeable quinone, comparable to Qa in the photosynthetic reaction centre, is required for Complex I activity, since CoQ is not present in fully active preparations of the enzyme (Drose et al., 2002). Cluster N2 interacts paramagnetically with two semiquinone species: the fast-relaxing species QNf is only observed in presence of a membrane potential, whereas the slow-relaxing species QNs is also seen during uncoupled turnover (Yano et al., 2005); it is not known whether the two signals reflect two semiquinones in different binding sites or two states of the same molecule (Brandt, 2006). The short-range interaction with N2, that is situated in the peripheral arm, suggests that the quinone head-group is not deeply buried in the membrane, despite the extreme hydrophobicity of the long isoprenoid tail. The structure of the side chain of quinones used for reconstitution is specific for integrated NADH oxidation activity (Lenaz, 1998a) although short CoQ homologues and analogues can be used as direct electron acceptors from the enzyme (Estornell et al., 1993). The observation that the K_m of Complex I for the short homolog CoQ_1 in bovine heart submitochondrial particles is reversibly increased without any change in V_{max} after depletion of endogenous CoQ_{10} by pentane extraction (Fato et al., 1996) suggests that CoQ_1 may interact with the enzyme active site either directly or via bound CoQ_{10} entered into the site from the pool (or shared from the Complex I–Complex III super-complex, see Section 2).

The actual mechanism of CoQ reduction is not known, and models have been based mostly on necessity to accommodate a mechanism for proton translocation (Ohnishi and Salerno, 2005; Hirst, 2005; Yagi and Matsuno-Yagi, 2003; Brandt, 1997; Dutton et al., 1998) but not based on actual experimental evidence; Sherwood and Hirst (2006) observed no evidence for a reductant-induced oxidation of ubiquinol, therefore excluding a Q-cycle type of mechanism.

It is generally assumed that two electrons are delivered to CoQ by the same iron–sulphur cluster by two consecutive electron transfer reactions (Ohnishi and Salerno, 2005; Hirst, 2005).

The recent findings in our laboratory pinpointing two different classes of inhibitors on the basis of their opposite effects on oxygen reduction to superoxide during forward electron transfer in bovine heart submitochondrial particles (Lenaz et al., 2006a) allow the concept of two minimal schemes of electron transfer in Complex I. In a linear scheme the electron donor to oxygen is presumably FeS cluster N2, whose reduction is inhibited by stigmatellin whereas its re-oxidation is inhibited by rotenone. In a bifurcated scheme (Fig. 5), the first electron is delivered to CoQ with formation of semiquinone in a rotenone-sensitive way; semiquinone is then reduced to quinol by N2 in a stigmatellin-sensitive way. N2 is also the donor to oxy-
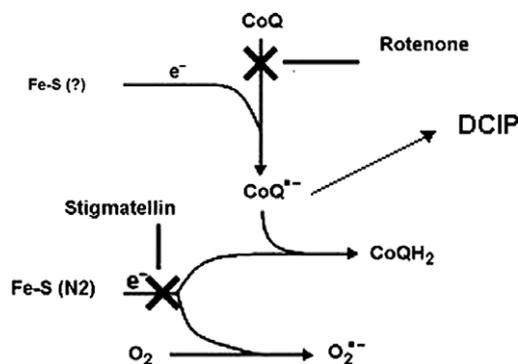
Fig. 5. Two-site hypothesis for electron transfer in Complex I to acceptors acting at the physiological site. Coenzyme Q is reduced by a two-step mechanism: an iron sulphur cluster (located upstream of center N2) is the donor of the first electron to CoQ, giving the semiquinone form. Semiquinone is completely reduced to quinol by one electron coming presumably from center N2. This scheme is supported by our findings on the Complex I inhibitors effect on superoxide production and DCIP reduction. The picture shows the sites of inhibition by rotenone and stigmatellin, the first acting on the first electron transfer step to quinone, the latter acting on the second step blocking electron transfer to semiquinone or to molecular oxygen.

gen; rotenone does not prevent delivery of one electron to N2 and then to oxygen, while stigmatellin prevents electron delivery to either ubisemiquinone or oxygen. The bifurcated scheme appears more in line with the position of the stigmatellin-inhibition site downstream with respect to the rotenone site, since the behaviour of stigmatellin as an inhibitor is shared by reduced quinone analogues (Degli Esposti, 1998).

A further confirmation of this scheme derives from the effect of inhibitors on reduction of the acceptor dichlorophenol indophenol (DCIP). Some DCIP is reduced at the level of FMN, since there is a component insensitive to DPI; another component is sensitive to DPI and must be reduced at the level of CoQ. In fact both hydrophilic and hydrophobic quinones enhance DPI-sensitive DCIP reduction. This last effect is still allowed in presence of stigmatellin but not in presence of rotenone (Lenaz et al., 2006a).

These findings demonstrate that DCIP is reduced at a site situated between the rotenone and the stigmatellin inhibition sites, a further indication for a split pathway of electrons at the CoQ binding site. According to the scheme presented in Fig. 5, DCIP would be reduced by ubisemiquinone, since its formation is rotenone sensitive but stigmatellin insensitive.

These findings are in contrast with findings in isolated Complex I (Kussmaul and Hirst, 2006; Galkin and Brandt, 2005) where FMN is considered the major electron donor to oxygen to form superoxide anion. In particular, accurate redox titrations of the electron donor and EPR study of the different redox centres (Kussmaul and Hirst, 2006) appear to exclude either FMN semiquinone or any FeS cluster as the source of superoxide, suggesting that the fully reduced flavin delivers one electron to oxygen and the other one to the chain of iron–sulphur clusters. The findings from our laboratory of two classes of inhibitors with respect to

superoxide generation (Lenaz et al., 2006a) are difficult to reconcile with a mechanism whereby flavin is the electron donor to oxygen. A possible explanation is that two sites for oxygen reduction exist in the complex, represented by flavin and an iron–sulphur cluster; the latter site would be predominant in membrane particles whereas the former one might be made more available after Complex I isolation. The role of super-complex organization in shielding/opening different sites in the enzymes cannot be overlooked.

The possible existence of two sites involved in CoQ reduction emerging from the study of Lenaz et al. (2006a) have to be reconciled with the linear pathway of electrons along the series of iron–sulphur clusters as demonstrated by the crystallographic study of Sazanov (Hinchliffe and Sazanov, 2005); our interpretation is not in contrast with the existence of a linear pathway, because the two electrons delivered to CoQ for its complete reduction could well be provided alternatively by two different clusters (or even by the same cluster) if a suitable conformational change occurs after the first electron delivery in order to provide a gating mechanism for the second electron.

4. Pathological implications

4.1. Of super-complex organization

The involvement of mitochondria in a variety of pathological aspects and in aging (Beal, 1992; Diplock, 1994; Götz et al., 1994; Lenaz, 1998b; Wallace, 2005; Balaban et al., 2005) has been largely ascribed to their central role in production of Reactive Oxygen Species (ROS) and to the damaging effect of ROS on these organelles. In particular, damage to mitochondrial DNA (mtDNA) would induce alterations of the polypeptides encoded by mtDNA in the respiratory complexes, with consequent decrease of electron transfer activity, leading to further production of ROS, and thus establishing a vicious circle of oxidative stress and energetic decline (Lenaz et al., 1999; Ozawa, 1997). This fall of mitochondrial energetic capacity is considered to be the cause of aging and age-related degenerative diseases (Lenaz, 1998b; Wallace, 2005; Ozawa, 1997; Lenaz et al., 2006b). This vicious circle might be broken by agents capable of preventing a chain reaction of ROS formation and damage, such as CoQ in its reduced form (Ernster and Dallner, 1995).

The observation that lipid peroxidation disrupts the aggregation of complexes I and III (see above) has profound patho-physiological implications, since ROS produced by the mitochondrial respiratory chain induce a progressive peroxidation of mitochondrial phospholipids (Ernster and Dallner, 1995), and in particular of cardiolipin (Paradies et al., 2000, 2002) in aging and ischemic diseases, with demonstrated decreased activity of both Complex I and III (Petrosillo et al., 2003; Paradies et al., 2004). It is tempting to speculate that under the above conditions a dissociation of Complex I–III aggregates occurs,

with loss of facilitated electron channeling and resumption of the less efficient pool behaviour of the free ubiquinone molecules. Although no direct demonstration exists yet, dissociation of super-complexes might have further deleterious consequences, such as disassembly of Complex I and III subunits and loss of electron transfer and/or proton translocation; we could not even exclude that the consequent alteration of electron transfer may elicit further induction of ROS generation. The observation that Complex III alterations prevent proper assembly of Complex I has therefore deep pathological implications beyond the field of genetic mitochondrial cytopathies.

Following this line of thought, the different susceptibility of different types of cells and tissues to ROS damage may depend, among other things, on the extent and tightness of super-complex organization of the respiratory chain, that is dependant on phospholipid content and composition of the mitochondrial membranes.

Mitochondrial DNA mutations have been consistently found in cancerous cells (Wallace, 2005; Carew and Huang, 2002); they have been found to be associated with enhanced ROS production, and ROS act both as mutagens and cellular mitogens (Klaunig and Kamendulis, 2004); thus the involvement of mtDNA mutations in cancer may well be of pathogenic importance (Wallace, 2005).

In a recent study we have shown that a cell line of a malignant thyroid oncocytoma, characterized by abnormal mitochondrial proliferation (Cheung et al., 2000), contains a mutation of mitochondrial DNA preventing expression of subunit ND1 (Bonora et al., 2006). These cells exhibit a dramatic decline of ATP synthesis supported by NAD-dependent substrates, while in the mitochondria isolated from these cells the Complex I activity is strongly depressed (Bonora et al., 2006). Accordingly, the cell line produces much higher amounts of ROS compared with a line derived from a non-oncocytic thyroid tumor (Stankov et al., 2006). We have also found (Sgarbi G. et al., unpublished) by blue-native electrophoresis of mitochondrial proteins from the oncocytic cell line, a complete absence of high molecular weight aggregates containing either Complex I or Complex IV, that are instead present in a control cell line from a non-oncocytic thyroid tumour. If the absence of super-complexes comprising Complex I is in line with its disassembly due to lack of ND1, the absence of aggregated Complex IV must be a secondary phenomenon, possibly due to the alteration of the lipid environment by the excessive ROS production. It is relevant to quote the recent finding (Koopman et al., 2005) that Complex I defects induce mitochondrial proliferation as a consequence of increased ROS production. It is tempting to speculate that the abnormal mitochondrial proliferation characteristic of these tumours is due to stimulation by ROS of mitochondrial proliferation.

Is the existence and role of super-complexes compatible with the interpretation of the beneficial effects of exogenous CoQ_{10} administration on bioenergetic grounds? CoQ_{10}-treatment of HL60 cells resulted in incorporation of func-

tional quinone which was proved to induce a proportional increase of CoQ-dependent mitochondrial activities (Fernández-Ayala et al., 2005). Moreover, there is evidence, mainly indirect, that exogenous orally administered CoQ_{10} may be incorporated into mitochondria, at least in conditions of partial CoQ tissue deficiency, where it may enhance electron transfer and ATP synthesis, e.g. in genetic CoQ_{10} deficiency (Di Giovanni et al., 2001; Rotig et al., 2000), cardiac failure (Rosenfeldt et al., 2005), Parkinson's disease (Matthews et al., 1998; Shults et al., 2002; Beal, 2004) and Friedreich ataxia (Hart et al., 2005). The existence of I–III super-complexes where only inter-complex bound CoQ is active by channeling electrons from Complex I to Complex III is apparently incompatible with a dose-dependent effect of added CoQ_{10}; however the notion that inter-complex bound CoQ is in chemical equilibrium with CoQ in the pool (cf. Section 2.3.4.) is sufficient to explain the improved cell bioenergetics upon addition of exogenous CoQ.

4.2. Of electron transfer within Complex I

Knowledge of the mechanism of electron transfer in Complex I is a prerequisite for understanding the pathogenesis of diseases where this enzyme is involved. Moreover, the selective action of different inhibitors may also mimic alterations occurring at the protein level as a consequence of genetic or post-translational changes affecting the composition of different subunits of the complex.

Complex I malfunction is involved in aging (Lenaz et al., 2006b) and in several common neurological diseases of ageing. In ischemic brain injury the pathogenesis of the damage is certainly complex (Starkov et al., 2004) but it is probably related to excitotoxic stimulation of glutamate NMDA receptors induced by membrane depolarization following ischemic ATP depletion (Wieloch, 2001); the elevated levels of cytosolic Ca^{2+} resulting from NMDA receptor activation may deregulate mitochondrial physiology with activation of the permeability transition, osmotic swelling, cytochrome *c* depletion and ROS production. The production of ROS at the level of Complex I may have different origins: the increased levels of succinate in hypoxic conditions (Wiesner et al., 1988) may activate reverse electron transfer and NAD^+ reduction, a well known cause of ROS generation at Complex I (Starkov and Fiskum, 2003); moreover the loss of cytochrome *c* due to PTP opening (Kowaltowski et al., 1998; Maciel et al., 2001) keeps a reduced level of the electron carriers in Complex I thus favouring superoxide formation. The loss of pyridine nucleotides ensuing prolonged PTP opening (Di Lisa et al., 2001) might decrease the reducing pressure and contribute to *decreasing* ROS formation at Complex I, however under these conditions (high α-ketoglutarate, low NAD^+), ROS production may be stimulated at the level of α-ketoglutarate dehydrogenase, now recognized as a major site of ROS production (Andreyev et al., 2005; Starkov et al., 2004).

The involvement of mitochondrial dysfunction in the aetiology and pathogenesis of Parkinson's disease and other neurodegenerative diseases (Corti et al., 2005) is supported by a plethora of experimental evidence.

Parkinson's disease is a major neurodegenerative disease affecting 1% of European populations (Twelves et al., 2003) and characterized pathologically by Lewy bodies, cytoplasmatic eosinophilic inclusions in the *substantia nigra* neurons (Forno, 1996). The accumulation of aggregates of α-synuclein, a presynaptic protein of unknown function (Tofaris and Spillantini, 2005) is the main determinant of the Lewy bodies. Mutations of α-synuclein gene are known to induce a hereditary form of the disease by facilitation of its aggregation (Bennett, 2005). Also the non-mutated protein, however, may be induced to aggregate in the presence of post-translational modifications such as due to oxidative stress (Betarbet et al., 2001), nitration (Giasson et al., 2000), phosphorylation (Fujiwara et al., 2002) or truncation at the carboxyl end (Tofaris et al., 2003). The chemically modified forms are found aggregated in the Lewy bodies in a highly ubiquitinylated form (Tofaris et al., 2003).

The involvement of mitochondria in the pathogenesis of Parkinson's disease was first suggested by the effects of the drug MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). MPTP induces parkinsonism via inhibition of Complex I by way of its metabolite 1-methyl-4-phenylpyridinium (MPP^+) (Singer et al., 1987). More recently it was observed that rotenone, the best known Complex I inhibitor, may reproduce the histological features of Parkinson's disease, including selective loss of nigro-striatal dopaminergic neurons, and appearance of Lewy bodies positive to α-synuclein (Sherer et al., 2003a). Thus, Complex I inhibition is sufficient to trigger Parkinson's disease in the rat. Mitochondrial DNA mutations may be involved in the aetiology and predisposition to the idiopathic disease, since cybrids containing mitochondria from Parkinson's patients exhibit a reduced activity of Complex I (Swerdlow et al., 2001) and generate Lewy inclusion bodies (Trimmer et al., 2004).

A precise role of mitochondrial Complex I in the formation of Lewy bodies through α-synuclein aggregation is not yet defined: nevertheless the hypothesis is tenable that a primary mitochondrial dysfunction may lead to enhanced ROS production (Sherer et al., 2003a), triggering cell death mechanisms in dopaminergic cells (Tretter et al., 2004): indeed neurotoxins inducing parkinsonism, such as MPP^+ and rotenone, stimulate ROS production by Complex I (see Section 2.1.1.).

The reason why neurons in the *substantia nigra* are specifically affected is not clear: however the presence of dopamine in the nigro-striatal nerve terminals may be an additional factor stimulating an autocatalytic cascade of ROS formation. Oxidation of dopamine to dopaminochrome by superoxide may trigger a vicious cycle of oxidative stress through the reduction of dopaminochrome by Complex I to its semiquinone form and its re-oxidation

by oxygen to form additional superoxide (Spencer et al., 1998; Zoccarato et al., 2005). Indeed this mechanism has been proven for an adrenaline / adrenochrome cycle in isolated mitochondria (Bindoli et al., 1990; Genova et al., 2006).

Humans are exposed to a great number of Complex I inhibitors, since numerous insecticides and pesticides belong to this category (Degli Esposti, 1998; Okun et al., 1999; Lümmen, 1998). The aetiology of Parkinson's disease, though uncertain, may include chronic exposure to such compounds either in adulthood or during development (Barlow et al., 2004; Cory-Slechta et al., 2005), in association with genetic susceptibility (Elbaz et al., 2004; Jenner, 2001; Paolini et al., 2004; Benmoval-Segal et al., 2005); epidemiological studies (Li et al., 2005; Privadarshi et al., 2001; Baldi et al., 2003; Perier et al., 2003), the effects of Complex I inhibitors in animal models of the disease (Sherer et al., 2003b; Ossowska et al., 2005; Bovek et al., 2005; Schapira et al., 1998) and the observed involvement of Complex I defect in Parkinson's patients (Schapira et al., 1998; Schapira et al., 1989; Schapira, 1994; Haas et al., 1995; Reichmann and Yanetzky, 2000; Muftuoglu et al., 2004) support the idea that environmental exposures and genetic mitochondrial dysfunction may interact and result in neuro-degeneration. The ability of some of these inhibitors to elicit generation of excess oxygen radicals (see Section 2.1.1.) may aggravate the damage induced by inhibition of electron transfer. Thus, knowledge of the capacity of the long list of compounds acting as Complex I inhibitors to induce oxidative stress has important practical implications.

Among the inhibitors of Complex I, β-carbolines are formed from tryptophan and related compounds (Fekkes et al., 2001) and naturally occur in brain (Collins and Neafsey, 2000), particularly in the *substantia nigra* (Matsubara et al., 1993); N-methylated aromatic β-carbolines strongly resemble MPP$^+$, the toxic derivative of MPTP (see above) whose administration produces parkinsonism in both humans (Singer et al., 1987; Davis et al., 1979; Langston et al., 1983) and animals (Burns et al., 1983; Burns et al., 1984; Chiueh et al., 1984; Mayer et al., 1986; Sonsalla et al., 1987), and are as well neurotoxic: their injection into the *substantia nigra* of rats causes nigrostriatal toxicity strongly resembling that induced by MPP$^+$ (Neafsey et al., 1995). It was recently found that 2,9-dimethyl-β-carbolinium is a potent inducer of apoptosis in mouse neuroblastoma 2A cells, similarly to MPP$^+$, by similarly raising the production of ROS (Pavlovic et al., 2006); as a consequence, the dopamine levels were strongly depressed in the striatum in a dose-dependent manner. Although it is not known if the increase of ROS production is directly related to Complex I inhibition, waiting for studies in isolated mitochondria, these observations are very pertinent to the development of idiopathic Parkinson's disease.

Recently, Seo et al. (2006) and Richardson et al. (2007) reported that viral-mediated transgene expression of the yeast single-subunit NADH dehydrogenase (NDI1) can be used in cells and in animals to determine the relative contribution of Complex I inhibition in the toxicity of MPTP: NDI1 overexpression totally abolished the toxicity of the active metabolite of MPTP in SK-N-MC neuroblastoma cell cultures and, *in vivo*, protected mice from both the neurochemical defects in the nigrostriatal system and the behavioural deficits elicited by MPTP. Those findings demonstrated that the lack of the binding site for rotenone on NDI1 was sufficient to prevent the toxicity of MPP$^+$ and clearly identified inhibition of Complex I as a requirement for dopaminergic neurodegeneration produced by MPTP, which is the most widely used model for Parkinson's disease.

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The importance of plasma membrane coenzyme Q in aging and stress responses

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Abstract

The plasma membrane of eukaryotic cells is the limit to interact with the environment. This position implies receiving stress signals that affects its components such as phospholipids. Inserted inside these components is coenzyme Q that is a redox compound acting as antioxidant. Coenzyme Q is reduced by diverse dehydrogenase enzymes mainly NADH-cytochrome b_5 reductase and NAD(P)H:quinone reductase 1. Reduced coenzyme Q can prevent lipid peroxidation chain reaction by itself or by reducing other antioxidants such as α-tocopherol and ascorbate. The group formed by antioxidants and the enzymes able to reduce coenzyme Q constitutes a plasma membrane redox system that is regulated by conditions that induce oxidative stress. Growth factor removal, ethidium bromide-induced ρ° cells, and vitamin E deficiency are some of the conditions where both coenzyme Q and its reductases are increased in the plasma membrane. This antioxidant system in the plasma membrane has been observed to participate in the healthy aging induced by calorie restriction. Furthermore, coenzyme Q regulates the release of ceramide from sphingomyelin, which is concentrated in the plasma membrane. This results from the non-competitive inhibition of the neutral sphingomyelinase by coenzyme Q particularly by its reduced form. Coenzyme Q in the plasma membrane is then the center of a complex antioxidant system preventing the accumulation of oxidative damage and regulating the externally initiated ceramide signaling pathway.
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Keywords: Coenzyme Q; Plasma membrane; Aging; Oxidative stress

1. Introduction

Coenzyme Q or ubiquinone (CoQ) is constituted by a benzoquinone ring and a lipid side chain constructed with several isoprenoid units, the number of units being species specific. *Saccharomyces cerevisiae* has six isoprene units (CoQ_6), *Caenorhabditis elegans* CoQ isoform contains nine isoprenoid units (CoQ_9), and mammalian species have different proportions of CoQ_9 and CoQ_{10}. Redox functions of CoQ are due to its ability to exchange two electrons in a redox cycle between the oxidized (ubiquinone, CoQ) and the reduced form (ubiquinol, $CoQH_2$). This redox reaction can be driven either by the simultaneous transfer of two electrons in a single step, or by two sequential steps of one electron transfer through a partially reduced semiquinone intermediate. These redox forms allow CoQ to act as antioxidant but also as pro-oxidant mainly through the semiquinone intermediate (Nakamura and Hayashi, 1994).

CoQ is the only lipid antioxidant that is synthesized in mammals by all cells, and its biosynthesis is a very complex process which involves the participation of at least nine gene products in all the species studied (Johnson et al., 2005; Tzagoloff and Dieckmann, 1990). Most of the proteins encoded by these genes have not yet been purified and the regulation of this biosynthesis pathway is still largely unknown (Rodriguez-Aguilera et al., 2003; Turunen et al., 2004).

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CoQ transports electrons from mitochondrial respiratory chain complexes I and II to complex III and acts as antioxidant as well (Crane and Navas, 1997; Turunen et al., 2004). In addition, it functions as a cofactor for uncoupling proteins (Echtay et al., 2000), regulates the permeability transition pore opening (Fontaine et al., 1998), and it is required for the biosynthesis of pyrimidine nucleotides because it is the natural substrate of dihydroorotate dehydrogenase, an enzyme located at the inner mitochondrial membrane (Jones, 1980). CoQ also enhances survival of chemotherapy-treated cells (Brea-Calvo et al., 2006) and is required for the stabilization of complex III in mitochondria (Santos-Ocana et al., 2002). Since the application of the two-phase partition method to isolate high-purity plasma membrane fractions from mammal cells (Navas et al., 1989), it was confirmed that CoQ is present in all the cellular membranes including plasma membrane (Kalen et al., 1987; Mollinedo and Schneider, 1984). The presence of CoQ in non-mitochondrial membranes suggests not only an important role for CoQ in each membrane but also the existence of specific mechanisms for its distribution since the final reactions of CoQ biosynthesis pathway are located exclusively at the mitochondria in yeast (Jonassen and Clarke, 2000) and mammal cells (Fernandez-Ayala et al., 2005). CoQ is then driven to the plasma membrane by the brefeldin A-sensitive endomembrane pathway (Fernandez-Ayala et al., 2005).

CoQ contributes to stabilize the plasma membrane, regenerates antioxidants such as ascorbate and α-tocopherol, and regulates the extracellulary-induced ceramide-dependent apoptosis pathway (Arroyo et al., 2004; Kagan et al., 1996; Navas and Villalba, 2004; Turunen et al., 2004). NAD(P)H-dependent reductases act at the plasma membrane to regenerate CoQH$_2$, contributing to maintain its antioxidant properties (Navas et al., 2005). As a whole, both CoQ and its reductases constitute a trans-plasma membrane antioxidant system responsible of the above described functions (Villalba et al., 1998).

This review will focus on the functions of CoQ and its reductases in the plasma membrane, and its regulation under aging and stress conditions. We will also emphasize on the role of plasma membrane CoQ in the regulation of stress-induced apoptosis.

2. The antioxidant system of the plasma membrane

2.1. CoQ is reduced at the plasma membrane by NAD(P)H-oxidoreductases

The plasma membrane delimites the cell and different insults from the environment, like oxidative stress, can attack this structure. Diverse antioxidants are protecting the cell under these conditions, particularly ascorbate at the hydrophilic cell surface and both CoQ and α-tocopherol in the hydrophobic phospholipid bilayer. The analysis of CoQ at the plasma membrane has shown that both its reduced and oxidized forms can be detected, and also the

signal of its free radical (Kagan et al., 1998). An interchange of electrons could be possible between CoQ and other redox compounds such as ascorbate (Roginsky et al., 1998), DHLA (Nohl et al., 1997) and superoxide (Kagan et al., 1998) leading to regenerate CoQH$_2$, but the major source of electrons comes from different NAD(P)H-dehydrogenases (Beyer et al., 1996; Nakamura and Hayashi, 1994; Navarro et al., 1995; Takahashi et al., 1996). It has been demonstrated that the incubation of liver plasma membranes with NADH increases CoQH$_2$ levels with the concomitant decrease in oxidized CoQ (Arroyo et al., 1998), which acts through semiquinone radicals and also recycles vitamin E homologue in a superoxide-dependent reaction (Kagan et al., 1998). CoQ, but not the intermediate form of CoQ biosynthesis, is also reduced by NADH-dependent dehydrogenases in plasma membrane of *C. elegans* (Arroyo et al., 2006).

Several enzymes have been reported to function as CoQ reductases. These include the NADH-cytochrome b_5 reductase (Constantinescu et al., 1994) (Navarro et al., 1995; Villalba et al., 1995) and NADPH-cytochrome P450 reductase (Kagan et al., 1996), which are one-electron CoQ-reductases (Nakamura and Hayashi, 1994); and NAD(P)H:quinone reductase 1 (NQO1, formerly DT-diaphorase) (Beyer et al., 1996; Landi et al., 1997) and a distinct NADPH-CoQ reductase that is separate from NQO1 (Takahashi et al., 1992, 1996), which are cytosolic two-electron CoQ-reductases.

Both NADH-cytochrome b_5 reductase and NQO1 were demonstrated to act at the plasma membrane to reduce CoQ (De Cabo et al., 2004; Navarro et al., 1995). The NADH-cytochrome b_5 reductase has been found in the cytosolic side of the plasma membrane, where it is attached through a myristic acid and a hydrophobic stretch of aminoacids located at its N-terminus (Borgese et al., 1982; Navarro et al., 1995). As a CoQ-reductase, the solubilized enzyme displays maximal activity with CoQ$_0$, a CoQ analogue which lacks the isoprenoid tail, whereas reduction of CoQ$_{10}$ requires reconstitution into phospholipids (Arroyo et al., 1998, 2004; Navarro et al., 1995). NQO1 catalyses the reduction of CoQ to CoQH$_2$ through a two-electron reaction (Ernster et al., 1962). This enzyme is mostly located in the cytosol with a minor portion associated to the membranes, including plasma membrane, where has been recognized to be of importance to maintain the antioxidant capacity of membranes (Navarro et al., 1998; Olsson et al., 1993). It has been shown that this enzyme can generate and maintain the reduced state of ubiquinones such as CoQ$_9$ and CoQ$_{10}$ in membrane systems and liposomes, thereby promoting their antioxidant function (Beyer et al., 1996; Landi et al., 1997).

These two enzymes would contribute to the trans-plasma membrane redox system providing the electrons that are required to maintain its antioxidant properties (Villalba et al., 1998). NADH-ascorbate free radical reductase, a *trans*-oriented activity shows a strong dependency on the CoQ status of liver plasma membrane (Arroyo

et al., 2004) and NQO1 also contribute to the plasma membrane antioxidant system in different conditions such as oxidative stress and aging (De Cabo et al., 2004, 2006; López-Lluch et al., 2005). The yeast model shows a high level of analogies with mammalian systems (Steinmetz et al., 2002) allowing a genetic evidence of CoQ participation in plasma membrane redox activities that it is more difficult to assess in mammal cells. The *COQ3* gene of *S. cerevisiae* encodes for a methyl transferase in CoQ biosynthesis pathway, and yeasts harboring a *COQ3* gene deletion (*coq3Δ*) do not synthesize CoQ (Clarke et al., 1991). Yeast mutants *coq3Δ* displayed a significant decrease of NADH-ascorbate free radical reductase activity at the plasma membrane, and its full restoration was achieved when mutant cells were cultured either in presence of exogenous CoQ, or when transformed with a plasmid harboring the wild-type *COQ3* gene (Santos-Ocaña et al., 1998).

Based on these results it is possible to scheme a plasma membrane containing a trans-membrane electron transport system (Fig. 1) that drives electrons either from NADH-ascorbate free radical reductase, NQO1 or both to CoQ, which follows a cycle to $CoQH_2$ through the semiquinone radical. This compound is then able to recycle other antioxidants such as ascorbate and α-tocopherol. Both $CoQH_2$ and α-tocopherol also prevent lipid peroxidation chain reaction.

2.2. Oxidative stress modulates the CoQ-dependent antioxidant system of plasma membrane

The transfer of CoQ to the plasma membrane is an active process that depends on the endomembrane system after its biosynthesis in mitochondria (Fernandez-Ayala et al., 2005). It is interesting then to explore the mechanisms involved in the incorporation of CoQ in the plasma membrane.

The impairment of mitochondrial function with ethidium bromide, which causes mitochondria-deficient ρ° cells, induces an increase of CoQ levels at the plasma membrane resulting in enhanced trans-membrane redox activity (Gómez-Díaz et al., 1997). Trans-plasma membrane redox system is then increased to reoxidize cytosolic NADH and to export reducing equivalents to external acceptors, maintaining the $NAD^+/NADH$ ratio (Martinus et al., 1993), which is important to guarantee the genome stabilization through sirtuins (Sauve et al., 2006). This adaptation could be thus considered as a general response of eukaryotic cells to impaired mitochondrial function in order to regulate cytosolic $NAD^+/NADH$ levels (Larm et al., 1994). A similar interpretation would be considered for the improvement of both plasma membrane antioxidant system (De Cabo et al., 2004; Hyun et al., 2006a) and mitochondria efficiency (Lopez-Lluch et al., 2006) induced by caloric restriction, a nutritional model that extends life span by inducing sirtuins (Cohen et al., 2004).

$CoQH_2$ protects membrane lipids from peroxidation either directly or through the regeneration of α-tocopherol and ascorbate. However, CoQ is synthesized in all animal species and it is possible to postulate a regulatory pathway for $CoQH_2$ in order to provide an antioxidant protection of the cell. The oxidative stress induced by camptothecin in mammal cells increase CoQ biosynthesis to prevent cell death (Brea-Calvo et al., 2006), as it was observed under several kinds of oxidative stress (Turunen et al., 2004), suggesting that it represents an adaptation rather than the cause of the stress. According to this idea, enhanced biosynthesis of CoQ and/or CoQ-reductases could be responses evoked by cells for protection against oxidative stress.

Mammals can not synthesize α-tocopherol (vitamin E) and require Se. A severe chronic oxidative stress can be provoked by feeding rats with diets deficient in both nutrients (Hafeman and Hoekstra, 1977). After 3 weeks of deficient diet consumption, animals show markedly reduced levels of α-tocopherol in tissues, and display a dramatically increased Ca^{2+}-independent phospholipase A_2 activity (PLA_2), which may play a protective role in cells leading to increased metabolism of fatty acid hydroperoxides (Kuo et al., 1995).
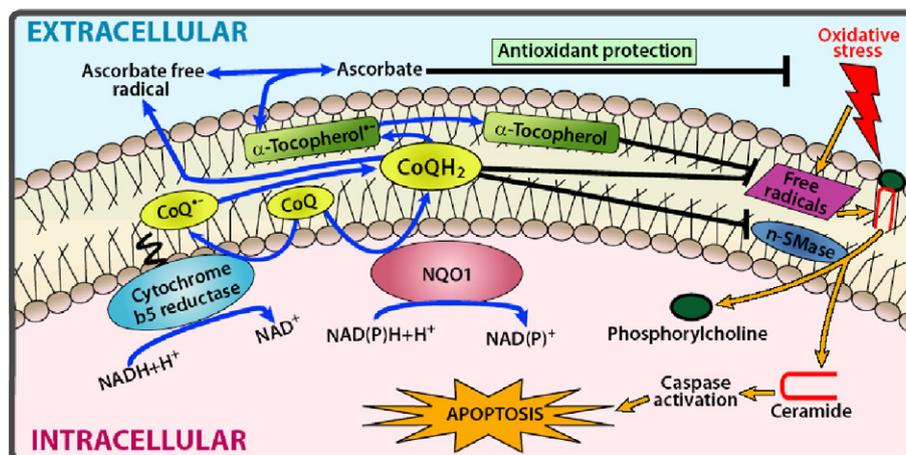
Fig. 1. An scheme of the plasma membrane redox system. It involves an antioxidant system and its role on the sphingomyelinase regulation. *Abbreviations:* n-SMase, neutral-sphingomyelinase; NQO1, NAD(P)H:quinone reductase 1.

Using this approach, an increase in both CoQ_9 and CoQ_{10} in liver plasma membranes was observed when α-tocopherol and selenium had reached minimum levels (Navarro et al., 1998, 1999). CoQ increase at the plasma membrane may be the result of enhanced biosynthesis and/or translocation from intracellular reservoirs such as the endoplasmic reticulum and mitochondria. These results would be supported because lower levels of total CoQ have been found in heart mitochondria isolated from vitamin E-deficient and vitamin E and Se-deficient rats (Scholz et al., 1997). An increase of CoQ biosynthesis under vitamin E and Se deficiency might be not enough to compensate for its accelerated consumption by oxidative degradation in the heart, an organ with high demand for CoQ utilization in oxidative metabolism. A transitional effect was observed when rats were submitted only to vitamin E deficiency, showing a milder adaptation to oxidative stress where antioxidants were induced earlier than the phospholipases (De Cabo et al., 2006).

Consistent with higher CoQ levels, deficiency was accompanied by a twofold increase in redox activities associated with trans-plasma membrane electron transport such as NQO1 and ascorbate free radical-reductase (De Cabo et al., 2006; Navarro et al., 1998). NQO1 was also increased in liver of rats fed with a diet deficient in selenium (Olsson et al., 1993), and it has been also shown that the expression of the NQO1 gene was induced in rat liver after 7 weeks of consuming a vitamin E and selenium-deficient diet (Fischer et al., 2001). However, the increase of CoQ and NADH-cytochrome b_5 reductase was earlier than NQO1 translocation to the plasma membrane indicating a timing of events leading to protect cells from oxidative stress (De Cabo et al., 2006). This is apparently a general aspect of response to endogenous oxidative stress (Bello et al., 2005b). Although the mechanisms involved in regulating the changes of CoQ concentration in the plasma membrane, and also the accumulation of its reductases are still elusive, it is postulated that the activation of stress-dependent signaling pathways such as mitochondrial retrograde signals would be involved.

2.3. The antioxidant system of plasma membrane is associated to aging process

There are a number of the key players on the plasma membrane that are affected by aging and age-associated diseases. The levels of antioxidants α-tocopherol and CoQ are decreased with age and elderly non-insulin-dependent diabetes mellitus (NIDDM) patients, suggesting that the pathogenesis of NIDDM could be associated with the impairment of an electron transfer mechanism by the plasma membrane redox system (Yanagawa et al., 2001). Oxidative damage to plasma membrane phospholipids in rat hepatocytes and brain increases with age and is retarded by caloric restriction (CR) (De Cabo et al., 2004; Hayashi and Miyazawa, 1998; Hyun et al., 2006b; López-Lluch et al., 2005). Increased levels of lipid peroxidation and decreased ratio

of docosahexaenoic acid to arachidonic acid are accompanied by a decrease in fluidity of the plasma membrane in aged rats (Hashimoto et al., 2001). These findings support the hypothesis that alteration of membranes by oxidative damage to their structural basic molecules, lipids and proteins, can be involved in the basic biology of aging.

Rats fed with a diet enriched with polyunsaturated fatty acids (PUFAn-6) and supplemented with CoQ_{10} show an increased life-span compared to those fed without the supplementation (Quiles et al., 2004). In these conditions, CoQ_{10} was increased in plasma membrane at every time point compared to control rats fed on a PUFAn-6-alone diet. Also, ratios of CoQ_9 to CoQ_{10} were significantly lower in liver plasma membranes of CoQ_{10}-supplemented animals (Bello et al., 2005a). These results clearly support the role of both CoQ and its content in plasma membrane in the regulation of aging process.

Data from our laboratories and others, provide support that the plasma membrane redox system is, at least in part, responsible of the maintenance of the antioxidant capacity during oxidative stress challenges induced by the diet and aging. The up-regulation of the plasma membrane redox system that occurs during CR decreases the levels of oxidative stress in aged membranes (De Cabo et al., 2004; Hyun et al., 2006b; López-Lluch et al., 2005). CR is the only reliable experimental model to extend life span in several mammalian models (Heilbronn and Ravussin, 2003; Ingram et al., 2006). CR extends life span of yeasts by decreasing NADH levels (Lin et al., 2004), which would connect this intervention to plasma membrane NADH-dependent dehydrogenases. CR modifies composition of fatty acid in the plasma membrane, resulting in decreased oxidative damage including lipid peroxidation (Yu, 2005; Zheng et al., 2005). More importantly, plasma membrane redox activities and also the content of CoQ, which decline with age, are enhanced by CR providing protection to phospholipids and preventing the lipid peroxidation reaction progression (De Cabo et al., 2004; Hyun et al., 2006b; López-Lluch et al., 2005).

3. CoQ participates in the regulation of apoptosis

3.1. CoQ of the plasma membrane prevent stress-induced apoptosis

The supplementation of mammal cells cultures with CoQ increases its concentration at the plasma membrane (Fernandez-Ayala et al., 2005), and also enhances cell growth (Crane et al., 1995). Also, molecular mechanisms that increase cell growth also increase trans-plasma membrane reductases (Crowe et al., 1993), most of them depending on the CoQ concentration in this membrane, as explained above.

Since the maintenance of cell population depends on the equilibrium among proliferation and cell death, it is important to understand the mechanisms that regulate cell survival. Hormones and growth factors are required to

prevent apoptosis that occurs with a mild oxidative stress (Ishizaki et al., 1995; Slater et al., 1995), and an increase of peroxidation levels in membranes (Barroso et al., 1997a; Ishizaki et al., 1995; Raff, 1992). As expected, the supplementation of cell cultures with various antioxidants in the absence of serum results in the protection against cell death (Barroso et al., 1997a,b). Steady-state levels of intra-cellular reactive oxygen species are significantly elevated in cells with low CoQ levels, particularly under serum-free conditions. These effects can be ameliorated by restoration with exogenous CoQ, indicating the major role of CoQ in the control of oxidative stress in animal cells (Gonzalez-Aragon et al., 2005).

Plasma membrane can be also a source of reactive oxygen species through the transport of electrons in the trans-membrane system (Hekimi and Guarente, 2003), which can be increased by antagonists of CoQ such as short-chain ubiquinone analogues and capsaicin that trigger apoptotic program starting at the plasma membrane (Macho et al., 1999; Wolvetang et al., 1996). This activity is different from the plasma membrane NAD(P)H oxidase of some cells such as neutrophils because it is currently accepted that this enzyme does not produce oxygen free radicals. The oxidase pumps electrons into the phagocytic vacuole, thereby inducing a charge across the membrane that must be compensated. The movement of compensating ions produces conditions in the vacuole conducive to microbial killing (Segal, 2005). CoQ is involved not only in the prevention of lipid peroxidation progression but also in recycling other antioxidants as indicated above. However, cells showing a higher concentration of CoQ in the plasma membrane (Gómez-Díaz et al., 1997) were more resistant to serum-removal oxidative stress-mediated apoptosis and accumulated lower levels of ceramide (Barroso et al., 1997b; Navas et al., 2002). We proceed then to analyze the role of CoQ of the plasma membrane in the ceramide signaling pathway.

3.2. CoQ inhibits neutral sphingomyelinase of the plasma membrane

The activation of a Mg^{2+}-dependent neutral-sphingo-myelinase (n-SMase) located at the plasma membrane has been recognized as one of the initial signaling events that take place during apoptosis induced by growth factors-withdrawal (Jayadev et al., 1995; Liu and Anderson, 1995). Serum deprivation induces a progressive increase of ceramide levels, which is then able to induce cell death after its intracellular accumulation (Barroso et al., 1997b; Jayadev et al., 1995; Obeid et al., 1993). These compounds then activate caspases, the general executioners of apopto-sis (Navas et al., 2002; Wolf and Green, 1999).

Mg^{2+}-dependent n-SMase was purified from plasma membrane showing that is highly inhibited by CoQ_0, an isoprenoid-free ubiquinone. This enzyme was also observed to be inhibited by CoQ_{10} in plasma membrane from pig liver through a non-competitive mechanism (Martin et al., 2001). These results suggest that CoQ may play a role

in the regulation of the n-SMase *in vivo*. The inhibition of n-SMase in the plasma membrane is carried out more effi-ciently by $CoQH_2$, and also depends on the length of the isoprenoid side chain (Martin et al., 2002) If the inhibition of plasma membrane n-SMase by ubiquinol has physiolog-ical significance, then endogenous levels of ubiquinol should also exert this regulatory action. Moreover, since endogenous CoQ can be reduced in plasma membrane by the intrinsic trans-membrane redox system, the activity of plasma membrane NAD(P)H-dependent dehydrogenases should also modulate the activity of n-SMase. This func-tion of CoQ occurs at the initiation phase of apoptosis by preventing the activation of the n-SMase in the plasma membrane through the direct inhibition of this enzyme and, as a consequence, the prevention of caspase activation (Navas et al., 2002). Fig. 1 shows not only the antioxidant function of CoQ and its reductases in the plasma mem-brane but also indicates the role of $CoQ/CoQH_2$, and probably lipid peroxides, in regulating the neutral sphingomyelinase.

Interestingly, different experimental studies have indi-cated that the exogenous treatment with CoQ stimulates the immune response (Bentinger et al., 2003). This latter effect and the inhibition of n-SMase by CoQ are factors to be considered that could be related to its beneficial effects on cells and organisms, beyond its participation in mitochondrial energy production or as antioxidant.

4. Conclusions and future directions

The plasma membrane redox system is important in cel-lular life because it prevents membrane damage but also reg-ulates the apoptosis signaling that starts at this membrane. It is currently known that this system responds to different type of stress increasing the concentration in the plasma membrane by new biosynthesis or translocation from the cytosol. More precisely, the study of biosynthesis regulation of both cytochrome b_5 reductase and NQO1, and its loca-tion to the plasma membrane is very important because is an essential enzyme not only for mammals but also to all eukaryotes. It is then important to analyze the signaling pathways responsible in the regulation of the plasma mem-brane redox system, and particularly its connection to mito-chondria, where coenzyme Q is particularly involved.

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The antioxidant role of coenzyme Q

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Abstract

A number of functions for coenzyme Q (CoQ) have been established during the years but its role as an effective antioxidant of the cellular membranes remains of dominating interest. This compound is our only endogenously synthesized lipid soluble antioxidant, present in all membranes and exceeding both in amount and efficiency that of other antioxidants. The protective effect is extended to lipids, proteins and DNA mainly because of its close localization to the oxidative events and the effective regeneration by continuous reduction at all locations. Its biosynthesis is influenced by nuclear receptors which may give the possibility, in the future, by using agonists or antagonists, of reestablishing the normal level in deficiencies caused by genetic mutations, aging or cardiomyopathy. An increase in CoQ concentration in specific cellular compartments in the presence of various types of oxidative stress appears to be of considerable interest.
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Keywords: Coenzyme Q; Lipid soluble antioxidants; Protein oxidation; Oxidative stress; Reactive oxygen species; Cellular organelles; Mevalonate pathway lipids

1. Introduction

Oxygen present in the atmosphere is the basis of our life on earth. Paradoxical is the fact that it is a very toxic substance under a number of conditions. Derivatives, such as hydroxyl and superoxide radicals, hydrogen peroxide and singlet oxygen may be formed and are called reactive oxygen species (ROS). These compounds appear not only in diseases but also under normal physiological conditions and interact with basic tissue components with consequences of disturbed function. Various types of antioxidant defense systems are, however, available in all organisms for limitation and elimination of these unwanted species.

Increased levels of free radicals are counteracted by antioxidants, but low concentrations of these compounds participate in redox signaling and by regulating gene expression they influence among others the activation and synthesis of antioxidants and other enzymes (Sen and Packer, 1996). Cellular effects elicited by minor amounts of ROS products, such as the generation of growth factors, production of hormones and modulation of tyrosine phosphatase are of great importance in cell cycle regulation, proliferation, differentiation and survival (Martindale and Holbrook, 2002). These radicals also participate in the regulation of immune response through the T cells by suppressing autoreactivity and development of arthritis (Gelderman et al., 2006).

2. ROS formation

In a number of enzymatic processes oxygen is reduced by one electron transfer to the superoxide radical $O_2^{-}\cdot$, hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH\cdot$) (Cadenas et al., 1992). The enzymes which belong to this group are the monoamine oxidase in mitochondrial outer membranes, acyl-CoA oxidase of peroxisomes, xanthine oxidase, microsomal NADH-cytochrome b_5 and cytochrome-P450 reductases and the NADPH oxidase in

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neutrophils. Mitochondrial electron transport is accounted for two-thirds of the cellular oxygen consumption and the observed limited leakage of electrons, 1–2%, is the largest contribution to the cellular $O_2^{-\cdot}$ and H_2O_2 production ([Papa and Skulachev, 1997](#)). Consequently, the steady-state concentration of superoxide radicals in the mitochondrial matrix greatly exceeds that of other cellular compartments. The functional activity of the mitochondria greatly influences the extent of ROS formation, low levels of ADP and high mitochondrial membrane potential gives high levels of ROS whereas high ADP levels and low membrane potential result in low production of ROS.

Several non-enzymatic pathways are known for ROS production, the most studied involves free or protein-bound transition metals, the metal-dependent reduction of H_2O_2 ([Halliwell and Gutteridge, 1984](#)). In practice, two metals are known as mediators, iron and copper. Singlet oxygen ($^1O_2$), an energized form of oxygen, is a common ROS product that is caused by light in the presence of sensitizers. Various peroxides and peroxide radicals are also richly produced during lipid and protein oxidation. A large number of studies deal with the interaction of superoxide radicals with nitric oxide, resulting in peroxynitrite, a highly cytotoxic compound that reacts with specific groups on proteins, e.g., thiols, transition metal centers and certain amino acids. Another common reaction in biological systems is that with carbon dioxide, leading to the formation of nitrogen dioxide and carbonate radical $CO_3^{\cdot -}$ ([Stamler et al., 1992; Pacher et al., 2007](#)).

3. Lipid, protein and DNA oxidation

Excess of free radicals affects the basic cellular constituents such as lipids, proteins and DNA and the damage caused is known as oxidative stress. Lipid peroxidation is a process studied in great detail and the mechanism is well characterized ([Ernster, 1993](#)). During the first phase, called initiation, an abstraction of a hydrogen atom from a methylene group of a fatty acid occurs presupposing that it has several double bonds ([Fig. 1](#)). This gives a carbon-centered alkyl radical (L$\cdot$) and rearrangement of the double bonds, diene conjugation. Reaction of L$\cdot$ with oxygen gives a peroxyl radical (LOO$\cdot$). In the second phase, called propagation, LOO$\cdot$ abstracts a hydrogen atom from an additional unsaturated fatty acid, leading to L$\cdot$ and lipid hydroperoxide (LOOH). LOOH can be reoxidized to LOO$\cdot$ which reinitiates lipid peroxidation. LOOH can also be reduced to an alkoxyl radical (LO$\cdot$) which again reinitiates lipid peroxidation, a process called "branching". Hydrogen abstraction from an adjacent polyunsaturated fatty acid by LO$\cdot$ gives L$\cdot$ and an alcohol (LOH) as the end product. Degradation of LOOH can follow several pathways giving hydrocarbons, alcohols, ethers, epoxides and aldehydes. Contrary to the other products, aldehydes such as malondialdehyde and 4-hydroxynonenal are long-lived, can diffuse from the site of their origin, react with and cross-link phospholipids, proteins and DNA. In the third phase, called ter-
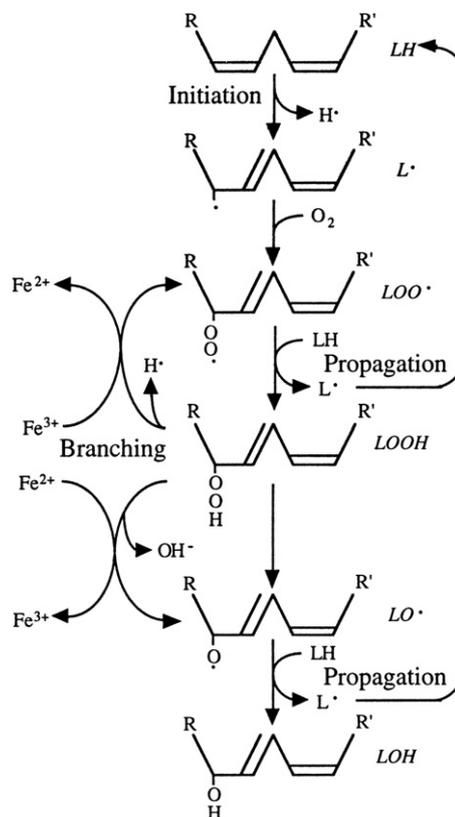
Fig. 1. Schematic steps in lipid peroxidation. LH, polyunsaturated fatty acid; L$\cdot$, carbon-centered radical; LOO$\cdot$, lipid peroxyl radical; LOOH, lipid hydroperoxide; LO$\cdot$, alkoxyl radical; LOH, alcohol end product.

mination, a non-radical product is formed after interaction between two radicals. The extent and velocity of lipid oxidation are dependent on the structural organization and composition of membranes and is consequently highly variable. When microsomal membranes are extracted with pentane and the extracted neutral lipids are substituted only with dolichol, which increases membrane fluidity, lipid peroxidation is elevated ([Jakobsson-Borin et al., 1994](#)). On the other hand, substitution with the membrane stabilizing cholesterol decreases the extent of oxidation. Lipid peroxidation damages phospholipids, but there are active mechanisms for repair involving removal of oxidized fatty acid with phospholipase A_2 and replacement with a new fatty acid by transacylation. Also, the lipid hydroperoxides formed during lipid peroxidation can be scavenged by phosphohydrolipid glutathione peroxidase.

Protein oxidation induced by free radicals occurs at the location of some specific amino acids of the individual protein since not all amino acids are sensitive to oxidation. Initiation takes place by reaction of hydroperoxide with a protein-bound transition metal, iron or copper, resulting in hydroxyl radicals at the metal binding site ([Stadtman, 1993](#)). This radical oxidizes the individual amino acid and the subsequent reactions that occur are similar to lipid peroxidation. The propagation phase can continue in different directions, within the protein, to another protein or to a lipid. In some processes, such as pulse radiolysis, no

transitional metal is required. Free radicals derived from lipid peroxidation are also able to mediate in protein oxidation. A usual finding is that products of lipid peroxidation, malondialdehyde and 4-hydroxynonenal bind covalently to amino acids, causing cross-linking and protein damage. In order to reestablish normal function the oxidized protein has to be replaced with newly synthesized forms. The oxidized entities can be removed by specific proteases that are able to hydrolyze the proteins to amino acids.

DNA oxidation elicited by ROS takes place in a similar manner to that of proteins and requires the presence of a bound transition metal (Halliwell and Aruoma, 1991). Mitochondrial DNA is preferentially exposed to damage since ROS products are present in high concentration at this location, there are no protective histones and the repair mechanism is limited. In the nucleus there are two possible pathways available for repairing the damage, removal and replacement of the appropriate nucleotide or removal of oligomeres of oxidized bases which are replaced by DNA polymerase and sealed by DNA ligase.

4. Distribution of CoQ

Biosynthesis of CoQ occurs in all tissues and cells in the animal organism and the lipid is present in all membranes (Turunen et al., 2004). The amount is specific for the membrane type which is apparent in comparison with the distribution of other membrane lipids (Table 1). The CoQ amount related to dolichol, cholesterol or α-tocopherol amounts is highly variable and is a consequence of the functional specialization associated with the individual membranes. From the point of view of antioxidant function it is notable that this lipid is present in all intracellular membranes. The high concentration in one organelle may reflect compartmentalization for different functions. In the inner mitochondrial membrane CoQ has at least four different functions, redox carrier, antioxidant, activator of uncoupling proteins and one of the compounds influencing the permeability transition pore. In this latter protein the associated lipid has to be in oxidized form, consequently,

this pool cannot be in cooperation with the portions participating in the respiratory chain or functioning as an antioxidant. The compartmentalization is emphasized by the fact that in *Caenorhabditis elegans* reduction of CoQ content by 60–70% does not decrease electron transport activity (Asencio et al., 2003).

The number of isoprenes on the side chain of CoQ is species specific, in human the chain consists of 10 isoprenes while in rat and mouse of nine isoprenes. A small portion, 2–7% of total CoQ in human tissues has solanesol as a side chain while in rat most tissues have about 10% and in some tissues 30% decaprenol. Various types of investigations concluded that this limited difference in chain length has no functional consequences and both CoQ9 and CoQ10 are equally efficient as antioxidant and redox carriers, and they are distributed evenly at the tissue and membrane levels (Zhang et al., 1996a). It appears that the trans-prenyltransferases are isoenzymes in different species and additionally to the main product they also synthesize shorter or longer polyisoprenes to a limited extent (Forsgren et al., 2004).

Polyisoprenoids are distributed in the central hydrophobic portion of the bilayer, between the phospholipid fatty acids (Valtersson et al., 1985; Lenaz et al., 1992). CoQ and dolichol in this position contributes to the destabilization of the membrane opposite to the action of cholesterol which is found among fatty acids on one side of the lipid leaflet and decreases membrane fluidity and permeability (Van Dijck et al., 1976). The benzoquinone containing end of CoQ possess an extensive mobility in the membrane between the two hydrophilic surfaces, an important property both for activity in the respiratory chain and for the antioxidant role. The chemical composition of a membrane is stabile without variation and this is also true for the membrane amount of CoQ. The number and arrangement of protein and lipid components set the upper limit necessary to keep the appropriate physicochemical state of the membrane. CoQ and dolichols increase fluidity and permeability while cholesterol stabilizes membranes and decreases fluidity. The effective ordering effect of CoQ is

Table 1
Coenzyme Q, dolichol, cholesterol and α-tocopherol in subcellular organelles of rat liver

Organelle	CoQ (μg per mg protein)	Dol+Dol-P (μg per mg protein)	Cholesterol (μg per mg protein)	α-Tocopherol (μg per mg protein)
Nuclear envelope	0.2	0.2	37.5	0.01
Mitochondria	1.4	0.09	2.3	0.04
Outer membranes	2.2	0.25	30	0.06
Inner membranes	1.9	0.009	5.0	0.04
Microsomes	0.2	0.4	28	0.07
Rough microsomes	0.2	0.5	16	0.04
Smooth microsomes	0.3	0.3	31	0.09
Lysosomes	1.9	4.7	38	0.18
Lysosomal membranes	0.4	0.6	6.1	
Golgi vesicles	2.6	1.7	71	0.48
Peroxisomes	0.3	0.8	6.4	0.02
Plasma membranes	0.7	0.8	128	0.008

CoQ, coenzyme Q; Dol, dolichol; Dol-P, dolichyl phosphate. Data taken from Low et al. (1992), Zhang et al. (1996b), Ericsson and Dallner (1993).

the consequence of the methoxy groups on the benzoquinone ring and also of the interaction of the quinone head groups with phospholipid molecules by hydrogen bonds. Both lack and excess of CoQ would disturb the balance between membrane stabilizing and destabilizing components and would be deleterious for basic functions such as permeability and enzyme activity. The consequence of this concept is that in the case of genetic deficiency of biosynthesis or in diseases such as cardiomyopathy the cell makes an effort to reestablish the lowered CoQ content in membrane using external sources. On the other hand, when the available spaces in the membrane are saturated with the lipid, external uptake is counteracted and the lipid is distributed in the luminal compartment or attached to soluble proteins (Low et al., 1992). This localization does not eliminate the possibility of functional activities such as acting as antioxidant for free radicals generated in the extramembranous space.

5. Antioxidant action of CoQ

There are four major groups of naturally occurring lipid soluble antioxidants, carotenoids, tocopherols, estrogens and coenzyme Q. The individual components in the groups vary greatly concerning distribution and effectiveness, e.g., in the group of vitamin E eight major compounds are operating, α-, β-, γ- and δ-tocopherols and -tocotrienols but in humans only α-tocopherol is effective as antioxidant. The liver contains a transfer protein that is specific for α-tocopherol and this compound is retained in the body while the other forms are rapidly excreted (Sato et al., 1991).

A large amount of data collected during the last 40 years by experiments on liposomes, mitochondria, microsomes, beef heart submitochondrial particles and lipoproteins of the blood demonstrated that CoQ in reduced form (named ubiquinol) is an effective antioxidant and inhibits lipid peroxidation. It was found that reduction of the lipid in submitochondrial particles with succinate or NADH abolished lipid peroxidation induced by ADP-Fe^{3+} but this protective effect was absent after removal of the neutral lipids with pentane extraction (Takayanagi et al., 1980). Reinsertion of CoQ into the extracted particles by using the pentane procedure decreased malondialdehyde formation after reduction of the lipid with succinate or NADH, demonstrating thereby the capacity of CoQ as an antioxidant. Pentane extraction also removes vitamin E and reincorporation of CoQ alone is able to restore the capacity to prevent lipid peroxidation (Forsmark et al., 1991). This proves again that CoQ itself in the absence of vitamin E has a pronounced antioxidant capacity. CoQ is our only endogenously synthesized lipid soluble antioxidant, it is mainly present in activated (reduced) form and its tissue amount is independent of the diet.

The effectiveness of CoQ as inhibitor of lipid peroxidation is based on its complex interaction during the peroxidation process. The primary action is the prevention of lipid peroxyl radicals (LOO$^\bullet$) production during initiation

(Fig. 2). $CoQH_2$ reduces the initiating perferryl radical with the formation of ubisemiquinone and H_2O_2. Additionally, it is possible that $CoQH_2$ eliminates LOO$^\bullet$ directly. It is also established that the reduced lipid effectively regenerates vitamin E from the α-tocopheroxyl radical, a favored reaction in comparison with the regeneration with ascorbate (Mukai et al., 1990).

Endogenous CoQ plays an important role in defending proteins from oxidation (Forsmark-Andree et al., 1995). Measured as protein carbonylation, in experiments where lipid oxidation also occurs, submitochondrial particles display intensive protein oxidation upon addition of ADP-Fe^{3+} and ascorbate (Table 2, Exp A). In the presence of succinate and antimycin the whole CoQ pool is reduced since antimycin inhibits Complex III and thereby the electron flow on the respiratory chain, which effectively prevents protein oxidation. Removal of CoQ by pentane extraction abolishes the effect of succinate–antimycin but the protection is restored after reincorporation of the lipid into the depleted particles. The CoQ action is a direct effect on the protein oxidation process. Addition of superoxide dismutase and catalase has no influence, demonstrating that superoxide radicals and hydroperoxides are not directly involved (Table 2, Exp B). The hydroxyl radical scavenger mannitol does not inhibit the formation of protein carbonyls. Both the phenolic antioxidant BHT and the iron chelator EDTA effectively counteract carbonyl production. The latter effect is the consequence of the iron dependency of the oxidation reaction.

CoQ is effective in preventing not only lipid peroxidation but also protein oxidation by quenching the initiating perferryl radical and functioning as a chain-breaking antioxidant, thus preventing propagation. There are several
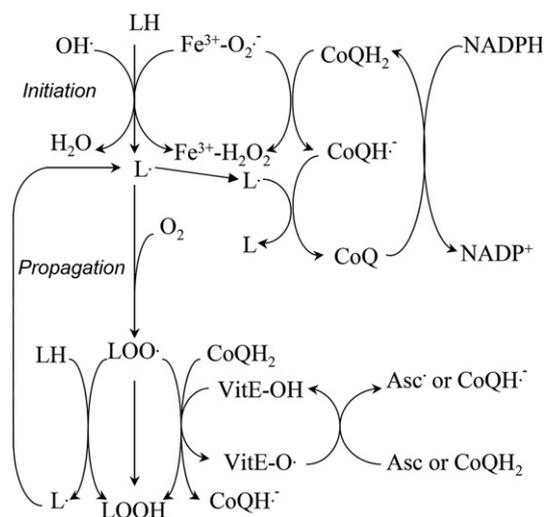
Fig. 2. Sites of action of CoQ on lipid peroxidation. LH, polyunsaturated fatty acid; OH$^\bullet$, hydroxyl radical; $Fe^3O_2^{\bullet-}$, perferryl radical; $CoQH_2$, reduced coenzyme Q; CoQH$^{\bullet-}$; ubisemiquinone; L$^\bullet$, carbon-centered radical; LOO$^\bullet$, lipid peroxyl radical; LOOH, lipid hydroperoxide; VitE-O$^\bullet$, α-tocopheroxyl radical; asc$^\bullet$, ascorbyl radical.

Table 2
Protein oxidation in submitochondrial particles

	Carbonyls (nmoles/mg protein)		
	None	ADP/ Fe^{3+}/asc	ADP/Fe^{3+}/asc + antimycin + succinate
Exp A			
Submitochondrial particles	1.5	13.7	1.3
Pentane extracted particles		14.5	13.6
Extracted particles, CoQ reconstituted		13.9	2.2
Exp B			
Submitochondrial particles	1.0	14.2	
+Superoxid dismutase		17.5	
+Catalase		17.0	
+Mannitol		13.7	
+BHT		1.1	
+EDTA		1.2	

Data taken from Forsmark-Andree et al. (1995).

mechanisms in protein oxidation and it appears that the most common is the direct oxidation of amino acid residues (Stadtman and Levine, 2000). To some extent, protein oxidation may also occur by lipid-derived free radicals and by breakdown products of phospholipid hydroperoxides, malondialdehyde and 4-hydroxynonenal (Rohn et al., 1998). These compounds link covalently to basic amino acid residues and in the latter case also to sulfhydryl groups causing intra- and intermolecular cross-linking. The sensitivity of different proteins to oxidative stress varies to a great extent, depending on their structure, composition and localization. CoQ is not protective against all types of oxidative damage. Peroxynitrate, which may be generated in tissues with an excess of nitric oxide and superoxide production, damage mitochondrial nicotinamide nucleotide transhydrogenase, and this oxidation is not prevented by CoQ (Forsmark-Andree et al., 1996). The close spatial relationship of CoQ to the neighboring membrane proteins is a main factor for its protective effect against protein oxidation.

Oxidative stress may damage DNA by initiating a series of metabolic reactions in the cell leading to activation of nuclease enzymes that cleave the DNA backbone. However, a more common event is the interaction of H_2O_2 with metal ions bound to DNA leading to generation of hydroxyl radicals. Base oxidation is commonly determined by measuring 8-hydroxy-deoxyguanosine (8-OH-dG). Determination of single-strand breaks, double-strand breaks or chromosomal aberrations are also employed as a measure of oxidative DNA damage. DNA oxidation in isolated mitochondria takes place in the presence of ADP-Fe^{3+} and ascorbate resulting in an elevated content of 8-OH-dG (Table 3) (Ernster and Dallner, 1995). Incubation in the presence of succinate and antimycin which maximize the ubiquinol pool, eliminate this oxidative damage. Similarly, the increased strand breaks caused by ADP-Fe^{3+} is greatly decreased by the endogenous ubiquinol. Thus, CoQ also plays a role as an antioxidant defending DNA

Table 3
Oxidative damage of DNA in rat liver mitochondria

Additions	DNA oxidation 8-OH-dG/10^5 dG	Strand breaks %
Control	117	6
ADP/Fe^{3+}/ascorbate	155	35
ADP/Fe^{3+}/ascorbate + antimycin + succinate	110	12

Data taken from Ernster and Dallner (1995).

which is of particular interest for mitochondrial DNA since this damage is not easily reparable.

In summary, the effectiveness of CoQ as antioxidant is derived from the fact that this compound interferes with lipid peroxidation both in the initiation and the propagation steps, contrary to the effect of vitamin E, which is a chain-breaking antioxidant and inhibits only propagation. Interference with protein and DNA oxidation is also documented. Lipid solubility and distribution in membranes is of great importance as hydroxyl and superoxide radicals formed in the membrane rapidly react with neighboring lipid and protein molecules which require the presence of an effective protective agent close to the radical production site. H_2O_2 diffuses out from membranes to the surroundings which is not the case for the other ROS products. An important property contributing to the antioxidant efficiency of this lipid is the large reducing capacity of the cell which is able to regenerate CoQ by reduction at all locations of the cell. In mitochondria, ubisemiquinone radical is formed during respiration which is effectively reduced to ubiquinol by the "protonmotive Q cycle" (Mitchell, 1975).

6. Enzymatic reduction

The amount of CoQ varies greatly in different tissues and subcellular organelles. In most cases the major part of the lipid is found in a reduced form. In spite of the fact

Table 4
Coenzyme Q amount and extent of reduction in human tissues

	CoQ10	
	μg/g tissue	% reduced
Heart	114	47
Kidney	67	73
Liver	55	95
Muscle	40	60
Brain	13	23
Pancreas	33	100
Spleen	25	87
Lung	8	24
Thyroidea	25	68
Testis	11	78
Intestine	12	93
Colon	11	83
Ventricle	12	59

Data taken from Aberg et al. (1992).

that during storage and isolation some autooxidation is expected, in most human autopsy material the proportion of the reduced form is the dominant species (Table 4). Autopsy material is probably not ideal for measurement of the steady state reduction of CoQ, however, the values are comparable with the values obtained on fresh tissues of rats. The amount of this lipid is substantially increased in disease and experimental conditions such as preneoplastic noduli in the liver, Alzheimer's and prion diseases in the brain, diabetes in the liver, peroxisomal induction and vitamin A deficiency in several organs and in chronic fluorosis in the brain. It is now established that oxidative stress is a dominating event in many pathological conditions and the assumption is that the increase in CoQ amount is an adaptive response, an attempt to create an adequate antioxidant defense against ROS products. Also, in these conditions the major part of the lipid is in reduced form, indicating that the reductive enzymatic mechanism is not a limiting factor. CoQ is present in blood plasma where it is bound to the three forms of lipoproteins (Dallner and Stocker, 2005). It is also increased in the plasma in hypercholesterolemia and hypertriglyceremia (Elmberger et al., 1991; Aberg et al., 1998). As in tissues, the major portion of CoQ in plasma is in reduced form, demonstrating that the enzymatic systems also at this location are effective (Yamashita and Yamamoto, 1997). In some pathological conditions the reduced/oxidized CoQ ratio may decrease somewhat but the main portion remains in reduced form (Yamamoto et al., 1998).

Several enzymes are proposed as reducing catalysts for CoQ. These include a cytosolic NADPH-dependent reductase (Takahashi et al., 1995) and the rotenone resistent DT-diaphorase (Beyer et al., 1996). It was also demonstrated that the FAD-containing homodimeric enzymes, within the family of pyridine nucleotide disulfide oxidoreductases, lipoamide dehydrogenase, glutathione reductase and thioredoxin reductase (TrxR1) are capable of reducing CoQ (Bjornstedt et al., 2004). Lipoamide dehydrogenase is present in the inner mitochondrial membrane but it is also located in the cytoplasm together with the other two oxidoreductases. The most effective of these three enzymes is TrxR1 which is a selenoprotein (Xia et al., 2003). Selenium deficiency lowers CoQ amounts in the liver by 50%, to a lesser extent in heart and kidney, but does not influence muscle (Vadhanavikit and Ganther, 1993). The extensive decrease in liver does not affect plasma membranes, which in spite of the deficiency of the lipid in the whole cell exhibit an elevated content (Navarro et al., 1998). However, the question of how the deficiency influences the redox state of the CoQ has not yet been investigated.

7. The antioxidant role in blood

In plasma the CoQ content in very low density, low density and high density lipoproteins is 1.2, 1.0 and 0.1 nmol/mg protein, respectively. The amounts are increased after dietary administration of the lipid to 3.2, 3.5 and 0.3 nmol/mg protein, respectively (Mohr et al., 1992). In all tissues the CoQ content is 6–10 times higher than that of vitamin E but in the blood its amount is only one-tenth of the main blood lipid soluble antioxidant, vitamin E. However, the very efficient reductive mechanism in the blood keeps CoQ in active form and it plays an important role in regeneration of vitamin E from the α-tocopheroxyl radical. It was shown that CoQ is preferentially utilized and more efficient in preventing LDL-oxidation than the other lipid soluble antioxidants in the blood, lycopene, β-carotene and α-tocopherol (Stocker et al., 1991). The preferential utilization of CoQ was also demonstrated in a liposomal system when a lipid soluble radical initiator was used (Shi et al., 1999). CoQ-enriched LDL was prepared from human subjects that were treated with dietary CoQ. The enriched LDL was more resistant to a number of oxidizing conditions than the native LDL (Mashima et al., 2001). It was also found that CoQ is able to counteract the prooxidant activity of vitamin E, observed when this latter antioxidant was employed alone (Thomas et al., 1996). When Cu^{2+} was used to induce oxidation, the effectiveness of CoQ was again documented (Tribble et al., 1994). Since oxidized LDL is considered to have a key function in the development of the atherosclerotic process, the effective intervention of CoQ with this oxidation is clearly of great interest.

Both mononuclear and polynuclear white blood cells contain appreciable amounts of CoQ, in opposite to red blood cells, which have very little, if any, of this lipid. Increase of CoQ content by *in vitro* incubation elevate DNA resistance to H_2O_2 induced oxidation but does not prevent DNA strand break formation (Tomasetti et al., 1999). Lymphocyte content of CoQ is doubled after *in vivo* administration of the lipid to human subjects with concomitant inhibition of oxidative DNA damage and enhancement of DNA repair enzyme activity (Tomasetti et al., 2001). Dietary administration increases the CoQ content of monocytes and lymphocytes but not in polynuclear cells (Turunen et al., 2002). Interestingly, in spite of the absence of uptake in polynuclear cells, these cells are still affected since during the treatment their vitamin E content is substantially elevated.

The major role of CoQ in the blood is considered to be as a protective antioxidant for lipoproteins. This view may change in the future. The lipid displays a strong antiatherogenic effect in apolipoprotein E-deficient mice fed a high-fat diet, however, not all the effects can be explained by the antioxidative action, as several oxidative stress markers did not decrease (Witting et al., 2000). It appears that other mechanisms are involved in the positive effects. In human studies dietary supplementation with CoQ resulted in a decrease of β2-integrin CD11b on the cell surface of monocytes (Turunen et al., 2002). The decrease of adhesion factors, like CD11b, reduces the recruitment of monocytes to the atherosclerotic lesions, an effective measure against further development of the disease. The accumulation of

arachidonic acid in the phospholipids of monocytes may be the result of inhibited cyclooxygenase activity and lowering of some inflammatory prostaglandins. Cosupplementation of CoQ with vitamin E in baboons significantly increases the antiinflammatory effect of vitamin E (Wang et al., 2004). In a healthy aged population of women, NK cell cytotoxicity is dependent on plasma CoQ10 concentration (Ravaglia et al., 2000). It appears that a number of beneficiary effects, other than the antioxidant protection, of CoQ in blood will be found in the future.

8. CoQ as prooxidant

It is known that electron leakage occurs during mitochondrial respiration leading to superoxide radicals and H_2O_2. Findings such as that respiration gives ubisemiquinone which reacts with oxygen, that antimycin increase electron leakage and that extraction of CoQ from mitochondria inhibits H_2O_2 production, leads to a plausible assumption that CoQ can promote oxidation in mitochondria. Part of the ubisemiquinone is produced at the outer section of the membrane, bordering the aqueous phase. This part may be subjected to autooxidation and generate superoxide radicals, followed by dismutation to H_2O_2. Hydrogen peroxide can also undergo reductive homolytic cleavage and form $OH^{\cdot}$ radicals (Nohl et al., 1998). These reactions demonstrate that CoQ under certain conditions may serve as a prooxidant. It appears that this sequence of events occurs preferentially in liposomal systems with high CoQ content but the probability for such a situation is much less in biological membranes. The capacity of enzymatic mechanisms to reduce the lipid at all cellular locations is in excess and in fact the whole or major part of CoQ is found, even after isolation, in reduced form. Also, oxidative processes are greatly limited in cellular membranes where phospholipids are organized around proteins and mixed with cholesterol. Additionally, several lines of observations indicate that not CoQ but other sites of the respiratory chain are responsible for the radical formation. Mitochondrial complexes have CoQ-binding proteins which counteract with the autooxidation process (Yu and Yu, 1981). Inhibition of Complex III with myxothiazol does not interfere with CoQ reduction, however, it decreases H_2O_2 formation (Nohl and Jordan, 1986). Also,

prevention of CoQ reduction by inhibition of Complex I does not eliminate electron leakage upon oxidation of NADH (Ramsay and Singer, 1992). In this situation this antioxidant within the mitochondrial environment does not appear to possess a prooxidant role, and outside of mitochondria the efficiency of the regenerating enzymes makes it improbable that oxidized CoQ formed will not be immediately reduced.

9. Stability and oxidative stress

There are several indications that under various pathological conditions CoQ in membranes is structurally modified and subjected to breakdown. The importance of these modifications is that the products may act as signaling molecules and influence a number of metabolic and synthetic pathways. The high degree of reduction of this lipid is not only a functional requirement but it is also a necessity for protection against oxidative damages (Forsmark-Andree et al., 1997). Submitochondrial particles incubated in the presence of ADP/Fe^{3+}/ascorbate exhibit a large increase in thiobarbituric acid reactive substances (TBARS) formation (lipid peroxidation) and lowering of CoQ amount, also leading to inactivation of NADH oxidase and succinate oxidase (Table 5, Exp A). When the same incubation is repeated in the presence of succinate and cyanide, which causes complete reduction of CoQ, no lipid peroxidation is observed and the CoQ content, NADH oxidase and succinate oxidase are at the control level. Liposomes consisting of cardiolipin (with linoleic acids) and CoQ upon incubation with ADP/Fe^{3+}/ascorbate lipid peroxidation suffer degradation of the major part of the CoQ (Table 5, Exp B). Phosphatidylcholine with saturated fatty acids (palmitic acids) is not a substrate for lipid peroxidation and consequently, after incubation with ADP/Fe^{3+}/ascorbate no TBARS are formed and no breakdown of CoQ occurs (Table 5, Exp C). Thus, during lipid peroxidation oxidative modification of oxidized CoQ takes place and the products are not able to function as respiratory chain components or antioxidants for prevention of protein and lipid oxidation. The products are more polar than the original lipid and potentially may exert biological effects. It is possible that various modified and breakdown products participate in a signaling process aiming to

Table 5
Lipid peroxidation and CoQ stability

	Additions	TBARS formation[a]	CoQ[a]	NADH oxidase[b]	Succinate oxidase[b]
Exp A Submitochondrial particles	None	0.3	4.8	1.4	0.7
	ADP/Fe^{3+}/asc	14.3	1.5	0.2	0.2
	ADP/Fe^{3+}/asc + antimycin + succinate	0.5	4.4	1.3	0.7
Exp B Liposomes, cardiolipin (tetralinoleyl)	ADP/Fe^{3+}/asc	17.3	0.2		
Exp C Liposomes, phosphatidylcholine (dipalmitoyl)	ADP/Fe^{3+}/asc	0.2	8.1		

Data taken from Forsmark-Andree et al. (1997).
[a] nmol/mg protein (Exp A), nmol/mg phospholipid (Exp B and C).
[b] µatoms O/min/mg protein.

influence CoQ biosynthesis. In diseases where oxidative stress is documented such as Alzheimer's and prion disease, diabetes and carcinogenesis, CoQ biosynthesis is increased (Turunen et al., 2004). The triggering mechanism in these conditions may be exerted by signaling metabolites.

10. Induction of biosynthesis

Lowered CoQ content in tissues may appear as a primary deficiency for genetic reasons, caused by aging or by damage of the biosynthetic system in diseases and toxic injury. The decrease may be extensive or mild and probably much more common than assumed today. In the case of cholesterol, the liver produces the lipid to supply other organs and one-third of the required daily amount is taken up from the diet. Consequently, cholesterol level in the blood is a mirror and diagnostic marker for the situation in organs. In the case of CoQ the rules are different. Under normal conditions all tissues and cells synthesize sufficient amount of the lipid which fulfills the cellular requirement and therefore no uptake from the blood occurs (Elmberger et al., 1987). The liver discharges small amounts of CoQ into the blood for lipoprotein protection but not for organ distribution. Dietary uptake is low and the uptake into organs from the blood is limited. Measurements of CoQ content in blood can be important and of diagnostic value, however, this value does not mirror the CoQ levels in various tissues. The present procedures do not allow for the taking of biopsies routinely and therefore we have no data to estimate the extent and distribution of CoQ deficiencies.

Primary CoQ deficiency is diagnosed when it leads to explicit disabilities (Rustin et al., 2004). It is probable, however, that there is a lower degree of deficiency in selected organs underlying a number of pathological conditions. During aging the lowering of CoQ in human organs is as much as 30–60% (Kalen et al., 1989). In common diseases such as in cardiomyopathy, cancer and degenerative muscle diseases there is an established and significant lowering of CoQ content in the target organ (Littarru et al., 1996). In these conditions decrease of CoQ is of importance since ROS products are continuously produced and the decrease of an effective endogenous antioxidant capacity is deleterious. Dietary administration is today the only possible approach to supply the cells with more of the lipid but because of the poor uptake this is not an optimal solution. The future aim is to find a pharmacological approach to increase the endogenous biosynthesis in the cell.

Ligands interacting with two nuclear receptors, PPARα and RXRα were found to be involved in biosynthesis of CoQ (Turunen et al., 2000; Bentinger et al., 2003b). RXRα is most often a partner in a heterodimeric receptor and its role can therefore be complex. Rodents treated with the peroxisomal inducers di(ethylhexyl)phthalate, clofibrate, salicilic acid and dehydroepiandrosterone increase CoQ synthesis and content in most organs but not in brain (Aberg et al., 1994). The effects elicited by these inducers are abolished in PPARα-null mice. Studies with RXRα-deficient mice demonstrated that this receptor is involved in the basic biosynthesis of CoQ, it is not required for the induction caused by peroxisomal inducers, but is required for the induction elicited by cold exposure. Since RXRα operates as a heterodimer in several nuclear receptors it is not known at present which is the receptor partner necessary for the observed effects. Clofibrate and several other fibrates are employed in human therapy in the treatment of hypertriglyceremia, which make it possible to test the effect of these drugs on levels of CoQ. The amount of the lipid, however, is not increased in humans after treatment, possibly because of the low levels of PPARα in human tissues (Aberg et al., 1998). It is a future task to find the appropriate agonist or antagonist for some of the transcription factors that specifically induce CoQ biosynthesis in human tissues.

Another possibility for induction of CoQ synthesis *in vivo* is to employ breakdown products or modified forms of this lipid. Catabolism of CoQ in tissues leads to one major product, having an unchanged substituted benzoquinone ring, with a short side chain which is carboxylated (Bentinger et al., 2003a). This compound is phosphorylated in the cells which make it water soluble, a requirement for transport in the blood and excretion by the kidney. Whether this or other minor breakdown products have some biological effects, has not yet been investigated. A decrease of CoQ amount in skin preparations was observed when subjected to ultraviolet irradiation (Podda et al., 1998). CoQ is broken down by UV-light and a large number of products have been identified upon irradiation of the lipid in solution. Among the products are a number of derivatives which have effects in tissue culture by stimulating CoQ biosynthesis. Isolation and identification of the products in this system is of considerable interest.

Dietary administration of CoQ10 to rodents is reported to cause an increasing amount of the endogenous CoQ9, indicating that the increased metabolism of the lipid caused by an external supply may activate a signaling mechanism that stimulates the synthetic pathway (Lenaz et al., 1993; Gomez-Diaz et al., 2003). As mentioned above, it is also possible that metabolites produced during oxidative stress are responsible for the increased synthesis of CoQ in several diseases.

11. Conclusions

The high efficiency of CoQ as a lipid soluble antioxidant is established because of its localization, effective reactivation and relatively high concentration. Additionally, it appears both to inhibit the initiation part and interfere with the propagation step of lipid and protein oxidation, a property not apparent in the case of other antioxidants. All tissues and cells are capable of synthesis of this lipid to such an extent that ensures sufficient local concentration without redistribution by the circulation and uptake from external sources. In conditions where free radical production is

part of the pathological process such as diabetes and Alzheimer's disease, the increase of tissue CoQ is considered to be one of the protective mechanisms. Uptake of dietary CoQ into the blood appears to be a major factor when the effects of this lipid are evaluated in pathophysiological conditions. It involves not only an increase of the antioxidant defense system but also stimulation of mechanisms related to the white cells and blood vessels. Changes in cytokine production, arachidonic acid metabolites, receptor distribution and endothelial functions will influence not only events in the circulation but also in tissues and organs. In tissue deficiency, in addition to external supply, stimulation of biosynthesis is required. This objective has not yet been attained but metabolic intermediates or modified metabolites interfering with transcription factors are good candidates for developing drugs which induce CoQ biosynthesis and improve the level of antioxidant defense.

Acknowledgements

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Coenzyme Q$_{10}$ – Its role as a prooxidant in the formation of superoxide anion/hydrogen peroxide and the regulation of the metabolome

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Abstract

Coenzyme Q$_{10}$ plays a central role in cellular bioenergy generation and its regulation. Closed membrane systems generate a proton motive force to create transient localized bio-capacitors; the captured energy is used for the synthesis of mitochondrial ATP but also for many other processes, such as metabolite translocations, nerve conduction and a host of other bioenergy requiring processes. Coenzyme Q$_{10}$ plays a key role in many of these sub-cellular membrane energy generating systems. Integral to this phenomenon is the prooxidant role of coenzyme Q$_{10}$ in generating the major superoxide anion/hydrogen peroxide second messenger system. This messenger system, largely but not exclusively, arises from coenzyme Q$_{10}$ semiquinone function; it contributes to the regulation of sub-cellular redox potential levels; transcription/gene expression control; is essential for modulated protein turnover and activation; mediates hormone and growth factor extracellular signaling. The regulated prooxidant formation of the superoxide anion/H$_2$O$_2$ second messenger system is essential for the normal physiological function of the metabolome. The normally functioning metabolome is the expression of a finely tuned dynamic equilibrium comprised of thousands of anabolic and catabolic reactions and all cellular signaling systems must be finely regulated. There is still much to be learnt about the up/down regulation of the H$_2$O$_2$ messenger system. The concept that superoxide anion/H$_2$O$_2$ cause random macromolecular damage is rebutted. The administration of antioxidants to quench the inferred toxicity of these compounds as a therapy for age associated diseases is unsupported by extant mammalian clinical trials and should be subject to serious re-evaluation. The role of ascorbic acid as a beneficial hydrogen peroxide prodrug is discussed.
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Keywords: Coenzyme Q$_{10}$; Prooxidant; Superoxide anions; Hydrogen peroxide; Second messenger; Metabolome

1. Introduction

This paper, in the main, focuses on the formation of superoxide anion and hydrogen peroxide and its role as a second messenger. The essential prooxidant role of coenzyme Q$_{10}$ and other superoxide anion/H$_2$O$_2$ generating systems is discussed. We continue to elaborate the thesis that the concept that superoxide anion/H$_2$O$_2$ are toxic products which randomly damage macromolecules and must be quenched by interventionist antioxidant therapy is flawed. On the contrary, there is an essential physiological require-

ment for superoxide anion/H$_2$O$_2$ formation, with H$_2$O$_2$ acting as a major second messenger required for normal metabolome function.

There is nothing more critical for all anabolic and catabolic cellular functions than an adequate, constant supply of bioenergy. Coenzyme Q$_{10}$ was shown by Crane et al. (1957), some 50 years ago to be a key component of the mitochondrial electron transport system. Soon after coenzyme Q$_{10}$ or its analogues were shown to be ubiquitous essential members of the electron transport and oxidative energy generating systems of bacteria and eukaryotic cells. Mitchell's (1974) revolutionary work concerning the mechanism of conservation of mitochondrial redox potential energy, overturning a 25 year phosphorylated intermediate

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hypothesis, owed much to his formulation of the Q cycle hypothesis (Mitchell, 1975). Essentially the Q cycle concept centred around mitochondrial proton motive force generated by vectorial separation of protons across impermeable biomembranes, with coenzyme Q_{10} playing a key role. It has been established that a wide range of biomembrane systems are energized via a redox process which create transient localized bio-capacitors, which are utilized by the metabolome (for review, Linnane et al., 2007b).

At about this time, Boveris, Chance and colleagues reported in a series of papers that the mitochondrial electron transport system through the agency of coenzyme Q_{10} semiquinone gave rise to high concentrations of superoxide anion, and in turn, hydrogen peroxide (for review Chance et al., 1979). It was calculated that 1–3% of inspired oxygen was converted to superoxide anion and in such amounts would be highly toxic to tissues. These reports appeared to support the free radical theory of aging first proposed by Harman (1956). A voluminous literature has arisen which has concentrated upon establishing the essential need for antioxidant systems to prevent random oxidative damage to cells and among other compounds, orally administered coenzyme Q_{10} functions as an antioxidant (Ebadi et al., 2001). A major problem with the concept of antioxidant therapy for the treatment of age associated systemic diseases is that there are no human clinical trials which support such a conclusion. By way of example, antioxidant therapy has been promoted for many years for the prevention and treatment of cancers, based on non-physiological in vitro studies (Ames et al., 1993). A wealth of data speaks to the contrary. Bjelakovic et al. (2004) have reported a meta-analysis of a series of antioxidant therapy studies (over 170,000 participants) and found no benefit for the treatment/prevention of gastrointestinal cancers or any effect on participant mortality. These studies included the administration of tocopherol, ascorbic acid, selenium and β-carotene in various combinations (for discussion, Linnane et al., 2007a,b). Similarly Miller et al. (2005) following meta analyses of 135,967 participants in vitamin E supplementation trials concluded that high doses of 400 IU/day may increase all cause mortality and should be avoided. It may be that any putative therapeutic antioxidant administered needs to be targeted specifically to appropriate sub-cellular sites and tissues/organs to have the desired effect of quenching over production of ROS, if it occurs. However, the current antioxidants do not appear to fit this situation. Recently Bailey et al. (2006) have reported that ascorbate promotes oxidative damage during surgical ischemia reperfusion.

In essence this short review considers two aspects of coenzyme Q_{10} functions, its role in energy generating mammalian oxido-reductase systems and simultaneously the prooxidant formation of small amounts of second messenger superoxide anion/H_2O_2. The formation of the prooxidant superoxide anion/H_2O_2 couple is so critical to overall metabolome function that it is also relevant to briefly consider its formation by non-coenzyme Q_{10}-dependent systems.

In considering/reviewing the encompassing biological function of coenzyme Q_{10} and prooxidants the subject matter can only be dealt with in a reductionist manner in this limited review. We have attempted to integrate a large body of work and publications emanating from diverse fields and as such include only two over-riding rather complex cartoon summary figures, relying on the text for clarification. Elsewhere in a series of papers the data summarized herein has been elaborated in more detail, we refer the reader particularly to Linnane et al. (2007b) for a more extensive treatment.

2. Superoxide anion and hydrogen peroxide formation

2.1. The role of coenzyme Q_{10} and sub-cellular signaling

Coenzyme Q_{10} acting through formation of its semiquinone is a major source of cellular and mitochondrial superoxide anion and consequently H_2O_2 formation. It also has a major role in mitochondrial energy generation actively participating in the establishment of the mitochondrial membrane's proton motive force ($\Delta p = \Delta \psi + \Delta pH$). Coenzyme Q_{10} occurs in most, if not all, cellular membranes and, it is again therein an important source of superoxide anion/H_2O_2, for example, the Golgi apparatus and lysosomal system contributing to the proton motive force established for these closed membrane systems.

We have earlier reported that coenzyme Q_{10} functions in the process of gene regulation (Linnane et al., 2002a,b). This conclusion arose from administering coenzyme Q_{10}, in a placebo controlled trial, to patients for a period of 4 weeks prior to undergoing hip replacement surgery. Subsequent to surgery, vastus lateralis muscle specimens were analyzed using histochemical analyses, microarray gene display, differential gene display and proteome analysis technologies to establish the profound effect coenzyme Q_{10} had on muscle fibre type composition, gene expression and the protein expression profile of human skeletal muscle. Coenzyme Q_{10} is anchored in cellular membranes as a member of oxido-reductase systems from which superoxide anion and H_2O_2 will arise. To explain the far reaching effect of coenzyme Q_{10} administration we concluded that H_2O_2 acted as a second messenger moving through the cell to deliver the coenzyme Q_{10} redox message from a number of sub-cellular membrane locations. Thus the fluctuating redox poise of coenzyme Q_{10} within its various membrane oxido-reductase systems generates a fluctuating amount of superoxide anion and in turn second messenger H_2O_2 which acts to signal the necessary modulation of the sub-cellular micro environment activities required for the normal function of the metabolome. The overarching role played by coenzyme Q_{10} in the holistic regulation of cellular function (Figs. 1a and b) acting largely as a key source of H_2O_2 has been discussed in more detail elsewhere and later herein (Linnane et al., 2002b; Linnane and Eastwood, 2006; Linnane et al., 2007a).

a

b
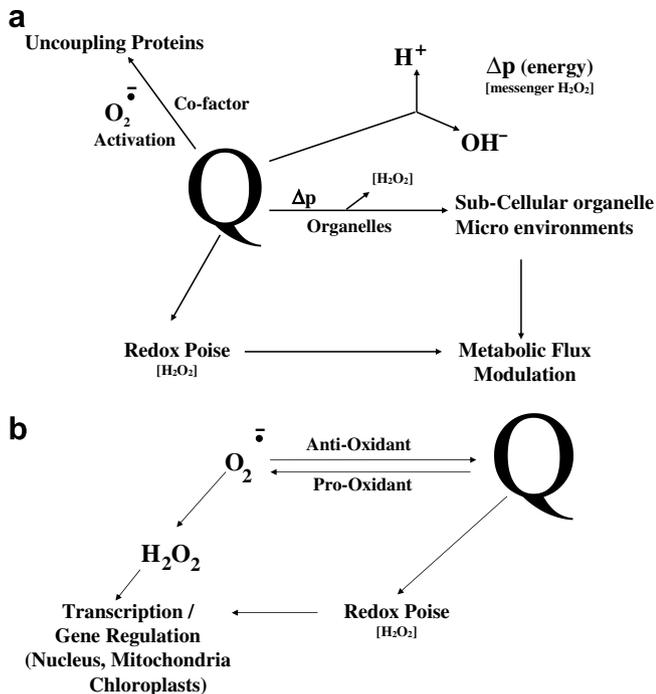
Fig. 1. Coenzyme Q_{10} oxido-reductase bioenergy system(s). (a) The cartoon summarizes the role of coenzyme Q_{10} oxido-reductase systems in energy generation and uncoupling and hydrogen peroxide second messenger generation regulating metabolic flux and redox poise sub-cellular microenvironments: the fluctuating oxidation/reduction status of coenzyme Q_{10} within the sub-cellular membranes results in fluctuating pH values and consequently the energy status of the various sub-cellular compartments creates a range of bioenergy microenvironments. Metabolic flux modulation: the fluctuating energy status of the various sub-cellular compartments will result in the fluxing of localized metabolic activity. (b) Prooxidant coenzyme Q_{10} regulatory signaling system(s). The cartoon summarizes the roles of coenzyme Q_{10} and H_2O_2 in the regulation of transcription and gene expression. For chloroplast details refer Pfannsch-midt et al. (1999) and Linnane and Eastwood (2004, 2006) Redox poise refers to fluctuating oxidation reduction states of coenzyme Q_{10} (and other redox systems) which constitutes a dynamic metabolome signaling system, largely modulated by H_2O_2 turnover.

2.2. Mitochondria

Central to a consideration of the roles of superoxide anion and hydrogen peroxide formation as related to their putative adverse effects and role as a second messenger system are the amounts formed under physiological conditions. Early reports suggested that 1–3% of inspired oxygen was converted to superoxide anion (Chance et al., 1979). These papers set the stage for future interpretations of the putative toxicity of metabolically generated oxygen radicals; indeed 1–3% of inspired oxygen converted to superoxide anion and H_2O_2 would be potentially physiologically catastrophic. A voluminous literature extending from the 1970's to the present day, has grown describing the toxic effects of excessively high concentrations of H_2O_2 on enzyme systems and cultured cells. However hydrogen peroxide is not a toxic compound, except at non-physiological unrealistically high concentrations; the common experimental use of millimolar concentrations

has lead to extrapolations and misinterpretations of the physiological role of superoxide anion and H_2O_2. It has erroneously become conventional knowledge in the field of antioxidant studies that excessively high levels of superoxide anion and H_2O_2 are produced in vivo as a by-product of energy metabolism and as such they are highly toxic to cells and that it is essential that they be quenched by antioxidants as rapidly as possible.

More recently the original, and much of the subsequent published data, emanating from many laboratories has since been shown to be interpretively misleading, in that normal (uninhibited) respiring mitochondria produce only very low, trace levels of superoxide anion and H_2O_2 (Nohl et al., 2001, 2005) confirmed and further elaborated by St-Pierre et al. (2002). St-Pierre et al. (2002) reported that the studies of Boveris, Chance and colleagues over estimated the amount of superoxide anion and H_2O_2 formed by mitochondria by about two orders of magnitude; the early estimate being about 10 nM H_2O_2/mg mitochondrial protein/min versus actual 0.1 nM H_2O_2/mg mitochondrial protein/min.

The St-Pierre paper is a notable contribution in its dissection of the topology of mitochondrial superoxide anion production; most significantly these authors reported that isolated rat skeletal muscle mitochondria respiring on complex I/III substrates release superoxide anion into the matrix while complex II/III substrates release superoxide anion into the medium. This vectorial synthesis of superoxide anion, extrapolated to cells would indicate that the resultant H_2O_2 formation enables it to act as a mitochondrial second messenger signaling to both nuclear and mitochondrial genomes. This signal would reflect the extant metabolic state of the mitochondrial organelle and its temporal requirement for appropriate nuclear and mitochondrial gene expression and metabolome modulation. Another notable feature of this paper is that heart muscle and skeletal muscle mitochondria oxidizing palmitoyl carnitine, vectorially release H_2O_2 differently. Isolated heart mitochondria oxidizing fatty acids (the heart's main energy substrate) release superoxide anion into the mitochondrial matrix while muscle mitochondria release it into the medium. These observations perhaps reflect that different tissue metabolisms have a requirement for different metabolic regulatory signaling messages.

Studies of knock out mice and superoxide dismutases provide some further insight on the role played by the superoxide anion/H_2O_2 couple. Construction of homozygous transgenic mice, null (−/−), for Mn SOD resulted in neonates being severely affected. Such animals as were born (no data were presented on prenatal deaths) died within a few days of birth as a result of severe cardiopathy, neurological and other pathological changes (Melov et al., 1998; Wallace, 1999). However, the interesting finding with this mouse model was that apparently only nuclear encoded proteins imported into the mitochondria from the cytosol were oxidatively damaged, as exemplified particularly by the Fe–S centre enzyme aconitase and nuclear

encoded complex II proteins of the electron transport chain. There was little effect on complex I, III and IV activities which require mtDNA encoded proteins for activity and it may be concluded therefore that mtDNA was not significantly oxidatively damaged in Mn SOD $(-/-)$ animals. In any event, our interpretation of these SOD $(-/-)$ animal results, is to suggest that such a major disruption to the H_2O_2 messenger signaling system would be expected to have a catastrophic outcome. Mitochondrially generated H_2O_2 is required for normal cell function hence Mn SOD null transgenic mice have no real survival value. We have elsewhere discussed the metabolic consequences of transgenic mice manipulations of cytosolic Cu/Zn SOD (Linnane and Eastwood, 2004; Linnane et al., 2007b). We concluded that experimental manipulations of Cu/Zn SOD leading to excessive depletion, or over production, of cytosolic essential second messenger H_2O_2 is detrimental to metabolome regulation and to cell function.

2.3. Lysosomal system/Golgi apparatus/secretory granules system

The early endosome arises from the plasma membrane and a proton translocating system functions to lower the internal pH of this membranous inclusion from about pH 7.4 to 6.2. This pH lowering process continues through to the late endosome (pH 5.3) and finally the lysosome (pH 5.0). Gille and Nohl (2000) have described a lysosomal redox chain. NADH was identified as a substrate for the system with a cytochrome b_{559}, a flavoprotein and coenzyme Q_{10} as components. Proton translocation into the strongly acidic lumen of the lysosomes through the agency of coenzyme Q_{10} was reported. The formation of superoxide anion was also described.

The early Golgi apparatus as it arises from the E.R. has an internal pH of about 6.7 progressing through to 5.4 at the secretory granule interface; it has been shown to contain a coenzyme Q_{10} oxido-reductase (Barr et al., 1984; Crane et al., 1994a) which will contribute to the acidification process and again superoxide anion H_2O_2 will be formed to function in a signaling mode.

2.4. Plasma membrane

Embodied within the plasma membrane are a number of complex signaling systems for the regulation of the cellular metabolome. There are two plasma membrane oxidoreductase systems (CNOX and Nox) which give rise to the superoxide anion/H_2O_2 system. The plasma membrane systems generation of H_2O_2 is incorporated into Fig. 2.

NADH oxido-reductase complex (later denoted CNOX, constitutive NADH oxidase) was first recognized as a coenzyme Q_{10} flavoprotein–cytochrome b_5 system localized on the outer face of the plasma membrane by Crane and colleagues some years ago. The activity of this system is quite low unless cells are exposed to extracellular growth promoting effectors, e.g., platelet derived growth factor

(PDGF) and epidermal growth factor (EGF). There are few details extant on the mechanism of activation of the CNOX system by the extracellular effectors. Crane et al. (1994b) proposed that the increased induced CNOX activity resulted in the formation of H_2O_2 which acted as a second messenger for the regulation of cell growth. The significance of the finding of a functional prooxidant activity of plasma membrane coenzyme Q_{10} as reported by Crane and associates has been largely overlooked with a focus centred on the putative antioxidant role of coenzyme Q_{10}. Perhaps the antioxidant/prooxidant role of coenzyme Q_{10} might be reconciled, for example, by consideration of the role of ascorbate acting as a H_2O_2 generating prodrug (Chen et al., 2005; Linnane et al., 2007b). In such a consideration the NADH CNOX–CoQ_{10} system functions to reduce extracellular ascorbate radical thus enabling ascorbate to be recycled, to continue in turn, to produce the H_2O_2 second messenger as part of the overall regulation of the metabolome (Crane and Low, 2001). Erythrocyte membrane studies add some weight to such a suggestion. Low et al. (1995) have reported the activation by H_2O_2 of erythrocyte glycolytic enzymes aldolase, phosphofructokinase and glyceraldehyde-3-phosphate dehydrogenase. These enzymes when bound to the cytoplasmic domain of intrinsic Band 3 protein of the erythrocyte membrane are inactive. Hydrogen peroxide (or ferricyanide resulting in H_2O_2 formation) treatment of the cells leads to activation of a tyrosine kinase which phosphorylates tyrosine residues of the intracellular domain of Band 3 resulting in the release of the three enzymes and their co-ordinate activation. The authors conclude that a plasma membrane electron transport system mediates the prooxidant formation of H_2O_2. Subsequently May and Qu (1999) reported that intracellular ascorbate was a key component in the generation of H_2O_2 by erythrocytes.

The CNOX system can be readily envisaged as concerned with cellular energy generation. NADH plasma membrane oxidation with oxygen as terminal acceptor, together with the mitochondrial bioenergy system and cytosolic glycolysis interactively contribute to cellular energy maintenance (Lawen et al., 1994). NADH is a major substrate required for mitochondrial energy generation while NAD^+ is required to maintain glycolytic ATP production. Lowered mitochondrial respiratory activity due to the aging process or its elimination in respiratory deficient ρ^0 cells is compensated by the upregulation of the plasma membrane NADH oxidizing systems to maintain NAD^+ levels and glycolytic activity (Larm et al., 1994; Kopsidas et al., 2000). This system can also provide other cellular needs for NAD^+, for example, as required by the sirtuin family of gene regulators. Caloric restriction (CR) increases average rodent life expectancy by as much as 50%; human epidemiological studies suggest that dietary CR can also increase average human life expectancy (Willcox et al., 2006). CR will result in the downregulation of overall metabolic pathways due to a less demanding metabolic substrate load. This proposition finds support from

Superoxide Anion / Hydrogen Peroxide:
**Redox Regulation of Gene Expression and Cellular Metabolism**
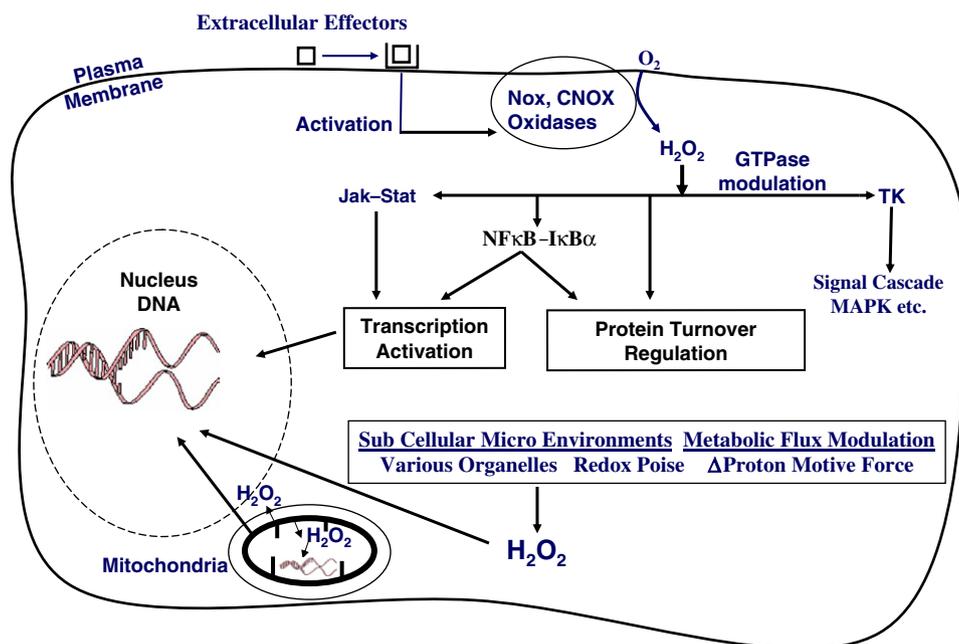
Fig. 2. Superoxide anion/hydrogen peroxide: redox regulation of gene expression and cellular metabolism. The cartoon summarizes an overview of the specific locales of hydrogen peroxide generation and its second messenger functions as a regulator of protein turnover and transcription/gene expression and the overall metabolome, as described in the text. Jak–Stat: Janus kinases–signal transducers and activators of transcription. Tyrosine kinase (TK). Mitogen activated protein kinase (MAPK).

the reported upregulation of the sirtuin family of NAD^+ -dependent histone deacetylases, which function to silence gene expression. Crane and Low (2005) have recently invoked the plasma membrane oxidase systems as sirtuin activity regulators by dint of being major sources of cytosolic NAD^+. Again superoxide anion/H_2O_2 formation will play a key role in the regulation of such systems and presumably in this instance, gene silencing.

The second plasma membrane NADPH oxidase system (Nox) does not contain coenzyme Q_{10} and may be subdivided into essentially two classes. One class is neutrophil/macrophage located, and produces high concentrations of superoxide anion/H_2O_2 (mM) functioning to kill sequestered bacteria. The second class, occurs probably in all cells and produces superoxide anion/H_2O_2 in the non-toxic nanomolar signaling range (for review, Linnane et al., 2007b). Most significantly the non-macrophage NADPH Nox activities are upregulated, analogously to CNOX, by a range of extracellular effectors (growth factors, cytokines and hormones) to produce H_2O_2 in a regulated manner presumably tailored to respond to the needs of the metabolomes characterizing the various tissues (for review, Werner, 2004). As a part of the activation process, the G protein Rac I, is recruited to become part of these NADPH oxidase complexes functioning to activate them to produce superoxide anion/H_2O_2 required for the downstream activation of Src protein phosphokinases which precipitate a regulatory protein phosphorylation cascade. The components involved and nature of the cascade, varies from one

tissue to another and will include, differentially, among others Raf, MEK, MAPK, MAPKK in turn regulating transcription factors Myc, Fos and Jun. On the other hand, as part of the overall regulatory activation/deactivation processes, protein phosphatases under cysteine/cystine redox cycling function to terminate and regulate the signals; also contributing will be catalase and glutathione peroxidase to the cellular equilibrium of the H_2O_2 signaling system.

3. H_2O_2 plasma membrane signaling specificity

The fact that the same extracellular effectors, e.g., PDGF, can stimulate both the CNOX and Nox systems to produce second messenger H_2O_2 raises the major unresolved problem of localized specificity. Does PDGF exposure lead to simultaneous H_2O_2 production by both systems, or is there some steric properties inherent in the plasma membrane which confers some selective effector regulatory specificity, and/or are there specific carriers (e.g., proteins) which move the H_2O_2 through the cell to elicit specific sub-cellular responses. There remains much to be learnt about H_2O_2 regulation and its second messenger role.

3.1. Proton motive force and cellular bioenergy

Membrane associated electrochemical energy can be calculated from measured membrane potential ($\Delta\psi$) and the

pH gradient across the membrane (ΔpH), whereby the sum of the values is equal to the proton motive force (Δp) generated across the membrane. This relationship is expressed by the following equation as:

$$\Delta p = \Delta\psi + \Delta pH$$

Cells are in the main comprised of a number of closed sub-cellular membrane systems of individual fluctuating proton motive forces. The immediately localized specific metabolic activity of the sub-cellular organelles will be under the control/regulation, of these energy fluxes. This information is developed more fully elsewhere (refer Linnane and Eastwood, 2004, 2006; Linnane et al., 2007b).

Membrane electro chemical energy (proton motive force) will vary, say, in millisecond time and serve to modulate sub-cellular organelle microenvironments and hence sub-cellular metabolic fluxes (Fig. 1a). The bioenergy generated by the proton motive force is used metabolically in the cellular regulation of ion, macromolecular and other substrate movements across membranes and in the case of mitochondria also for ATP synthesis. However coenzyme Q_{10} also functions as a source of superoxide anion to activate the uncoupler proteins (Echtay et al., 2002) and is an obligatory co-factor for uncoupling protein function (Echtay et al., 2000) as set out in Fig. 1a. Future studies are needed which consider the interactions between the various sub-cellular energy packets which together with glycolysis comprise the toti-cellular bioenergy state and function. In any event the superoxide anion/H_2O_2 upstream second messenger system, arising from the oxido-reductases activity of the cell's membranes and the associated proton translocations, play an essential role in the overall regulation of the cell's bioenergy status, its metabolic sub-cellular microenvironments and its particular metabolome (Fig. 1a).

Fig. 1b summarizes the roles of coenzyme Q_{10}/H_2O_2 in the regulation of transcription gene expression; the details of this cartoon are discussed later in the text.

4. Macromolecule oxidative changes and signaling

There is a voluminous literature reporting on oxidative damage to cellular macromolecular components by random, unregulated oxidation to cause deleterious damage leading to macromolecular and cellular dysfunction, thereby contributing to the aging process and age associated diseases. The process involving oxidative modification of macromolecules is much more subtle in its out workings.

5. Oxidatively modified proteins and regulated protein turnover

Protein degradation is exquisitely regulated; many proteins have half lives of only a few hours or less while others survive for days and weeks; the temporal turnover of these proteins is part of cellular metabolic regulation. Early studies of cellular proteolysis systems were concerned with the

cathepsins, a group of proteases with acidic optima of about pH5 encapsulated within lysosomes in order to protect the cell from random proteolytic action. The role of the cathepsins was supposedly to randomly hydrolyze proteins as part of the process of autophagy and endocytosis. Later the ubiquitin proteasome system was discovered and it is now recognised that cellular proteolysis is a regulated multi-system phenomenon which includes the lysosomal cathepsins.

The 26S proteasome enzyme complex is involved in the selection of proteins for degradation by the 26S unit. Ubiquitination of a protein is a three step process (E1, thioester bond formation; E2, transfer to protein sulphydryl group) with the third step catalysed by ATP-dependent ubiquitin protein ligases (E3's). It is necessary to appreciate that there are hundreds of E3's present in cells, specific to one or more proteins to be ligated to ubiquitin and thus tagged for 26S proteasome destruction. Further, polyubiquitination (4–50 ubiquitin residues) is required before final acceptance for hydrolysis by the 26S proteasome. The ubiquitin–ATP-dependent system which tags proteins for degradation by the proteasome is the major effector of protein turnover. Recently is has been recognised that ubiquitination is a key signal for targeting membrane intrinsic and extrinsic proteins for endosomal sorting and delivery to the proteolytic interior of the lysosome (for review Urbe, 2005).

Consider a commonly held view, that proteins are oxidized in an uncontrolled random process by superoxide anion/H_2O_2 (and NO and peroxynitrite not considered herein, refer Linnane et al., 2007b) and that such damage, unequivocally commits the damaged proteins to proteasome hydrolysis. This concept is no longer tenable, it is at best a gross over-simplification; we have discussed the processes more extensively elsewhere (Linnane et al., 2007b), a summary follows.

The oxidation of protein amino acid residues since their discovery some decades ago has been almost universally reported as leading to protein inactivation and requiring mandatory proteolysis to prevent their deleterious cellular accumulation. However it is clear that oxidatively modified proteins do not simply arise as the result of random oxidative damage (hydroxylations of various amino acid residues, sulphoxidation of methionines, nitrosylations of sulphydryl groups and so on). There are an increasing number of situations where free radical protein modifications can be shown to be part of normal cellular regulatory signaling activity.

The important nuclear transcription factor NFκB activity is regulated by superoxide anion formation. NFκB is maintained in an inactive form bound to the inhibitor IκBα. Following plasma membrane superoxide anion and H_2O_2 formation, induced by a range of cell effectors (e.g., cytokines, hormones), and regulated by Ras 1 (G protein), a transduction phosphorylation acts to phosphorylate IκBα and dissociate the complex leading to selective IκBα ubiquitination and proteasome destruction, in turn releas-

ing NFκB to translocate to the nucleus and function as a major transcription regulator (Figs. 1b and 2). This system teaches against the ready interpretation of cellular generating superoxide anion systems as being responsible for random protein damage.

It may be envisaged that the superoxide anion/H_2O_2 system is a universal messenger regulator of transcription, acting under the direction of all manner of cell effectors. The Jak/Stat system discovered by Darnell (for early review Darnell, 1998), is a major multifactorial transcription control system. The plasma membrane Nox oxidases produce superoxide anion/H_2O_2 messengers which activate the Jak–Stat system (Schieffer et al., 2000; Grote et al., 2005) again in response to a very similar range of extracellular factors (hormones, cytokines, growth factors) to that which activates the CNOX system (Figs. 1b and 2).

One of the most sensitive amino acids to oxidation is methionine, being converted to methionine sulphoxide (MetO), it is commonly cited as an example of random oxidative damage to proteins. Calmodulin (CM) function and its regulation by superoxide anion/H_2O_2 oxidation of specific methionine residues, is now well documented (Yin et al., 1999). The oxidation of only two specific methionine residues (Nos. 144 and 145) of calmodulin (there are seven) are involved in the process of downregulating, plasma membrane Ca^{2+}ATPase. The oxidation of other calmodulin methionines does not downregulate calmodulin–plasma membrane–Ca^{2+}ATPase activation (Yin et al., 2000). It has also been reported from the same laboratory (Sun et al., 1999) that methionine sulphoxide reductase, can act reductively, to restore the ability of oxidized calmodulin to regulate plasma membrane–Ca^{2+}ATPase. These results indicate that superoxide anion/H_2O_2 is functioning as part of the controlled regulation of the CM–PM–Ca^{2+}ATPase complex. Other examples including hemoglobin regulation and free radical reactions are presented elsewhere (Linnane et al., 2007b).

One of the complexities of H_2O_2 function is that it is theoretically, freely diffusible throughout the cell; but functionally that appears to be unlikely as the possibility would exist for the metabolome to simultaneously receive a multiplicity of messages which may be contradictory, self cancelling or over stimulatory. The H_2O_2 presumably must be locale specific or perhaps transported by some unknown regulatory system and also regulatory integrated with, at least, catalase and the glutathione system. The claimed toxic (pathophysiological) effects of superoxide anion/H_2O_2, may be the out workings of metabolic imbalance, such imbalance arising from dysfunctional hormone, growth factor, cytokine signaling among others. Clearly a plethora of disease states can arise from primary imbalances in cell signaling and does not conditionally arise as a consequence of induced macromolecular damage by superoxide anion/H_2O_2. Fig. 2 is a cartoon overview of the superoxide anion/H_2O_2 couple acting in their capacity as overall second messengers. It summarises their cellular production sites and their roles in mitogen activated pho-

sphokinase activations, protein turnover, sub-cellular metabolic redox modulation, mitochondrial and nuclear gene regulation and extracellular effector activations of the superoxide anion/H_2O_2 second messenger system.

6. Mitochondrial DNA

Age related decline in bioenergy capacity below a crucial threshold will obviously contribute to cellular malfunction. The development of mitochondrial tissue bioenergy mosaics with age, exemplified by null, low and normal cytochrome oxidase cell content has been stringently correlated at the single cell level, by our laboratory, with mtDNA deletions and the individual cell content of full-length functional mtDNA (Linnane et al., 1989; Nagley et al., 1993; Kovalenko et al., 1998; Kopsidas et al., 2000, 2002; Linnane et al., 2002a). The mtDNA deletion changes reported by us, mainly arise by replication error, due to the assymetrical nature of the heavy and light strand synthesis and the very large number of base pair repeats which mismatch during replication and result in mtDNA deletions. Cells whose mitochondrial oxidative phosphorylation function approaches zero due to severe mtDNA changes are lost from the tissue by apoptosis. The phenomenon of increasing age is fundamentally, cell loss from post-mitotic tissue. The cellular amount of mtDNA is under nuclear genome control so that if cells contain some full-length unmodified mtDNA it can be amplified back to normal levels, the various mtDNA deletions are eliminated and the cells rescued (Kopsidas et al., 2000). Cell loss occurs when little or no full-length mtDNA remains and therefore they become non-rescuable.

What role does oxidative damage play in inducing irreparable mtDNA dysfunction? Numerous early reports suggested that oxidative damage was a major contributor to increasing nuclear and more particularly mtDNA dysfunction. From such reports an error catastrophe hypothesis was proposed, whereby a vicious cycle of increasing oxidative damage to the mtDNA, lead to increased further oxidative damage and escalating mitochondrial bioenergy dysfunction. There is no extant evidence in support of such a view (Mansouri et al., 2006; Trifunovic et al., 2004, 2005). Urinary 8-oxy-deoxyguanosine was used as the exemplifier of age associated nuclear and mtDNA damage. However one of the major laboratories supporting the error catastrophe hypothesis subsequently reported that urinary measurements of 8-oxy-deoxyguanosine, as an estimation of increasing mtDNA (and nuclear DNA) damage, was unreliable and fraught with technical error and various confounding factors, leading to gross over estimation of oxidized mtDNA damage and uncertainty as to its interpretation (Helbock et al., 1998). The proposed dysfunctional formation of superoxide anion as a major cause of mtDNA damage and disease has yet to be demonstrated. On the contrary, recently Trifunovic et al. (2004, 2005) have reported that transgenic mice expressing an error prone mtDNA polymerase, accumulate substantial somatic

mtDNA mutations which are strongly correlated with a range of premature aging phenotypes and reduced life span. They did not observe any increase in so called oxidatively damaged products. These authors in agreement with our earlier human mtDNA studies conclude that oxidative phosphorylation dysfunction per se, arising from the replicatingly damaged mtDNA (and not oxidatively damaged mtDNA), is the primary inducer of the observed premature aging in the mtDNA mutator mice, and most particularly in a range of diseases normally associated with the aging process and observed in the mice.

7. Redox regulation of cellular metabolism and differentiation

The early work of Smith et al. (2000) on rat glial oligodendrocytes progenitor cell differentiation teaches that cellular redox poise regulates the process. Oligodendrocyte/astrocyte progenitor cells can be grown under conditions to establish a more oxidizing redox cytoplasmic environment, such conditions favour cell differentiation to oligodendrocyte or astrocyte formation. By contrast, a more reducing redox cytoplasmic environment favoured the maintenance of the progenitor cells. A particularly important aspect of these studies is that a range of naturally occurring physiological regulators function to modulate the redox state of cells, thus thyroid hormone and bone morphogenic protein 4 function to induce a more oxidizing cytoplasmic redox poise environment while basic fibroblast growth factor and PDGF exposure induce a more reducing cytoplasmic state. Different admixtures of hormones and growth factors favoring oxidation or reduction were used to manipulate cytoplasmic redox poise and cultures were thereby induced to predominantly self replicate or differentiate. Sundaresan et al. (1995) have reported that the response of rat vascular smooth muscle cells to PDGF, which includes a cascade of tyrosine phosphorylation, mitogen activated protein kinase stimulation and DNA synthesis was inhibited when PDGF induced H_2O_2 formation was blocked. There are numerous other examples of redox poise (H_2O_2) regulation playing a major role in cell differentiation (refer Linnane et al., 2007b).

8. The chimera of antioxidant (theory and) therapy?

The theme running through this short review is that a random antioxidant scavenging of superoxide anion (H_2O_2) would catastrophically derange their second messenger function which is essential for the regulation of major metabolome activities. The chimera of antioxidant therapy we have briefly considered elsewhere (Linnane and Eastwood, 2004). There is no compelling evidence from human clinical studies, conducted with sharp end points, to support the claims that the ingestion of small molecule antioxidants such as vitamins C, E, β-carotene and others prevent/ameliorate the development of age

associated human diseases presumptively arising from random oxidative damage to cellular systems (Linnane and Eastwood, 2004; Linnane et al., 2007b).

9. Ascorbic acid, a hydrogen peroxide prodrug

Vitamin C has long been promoted as an outstanding antioxidant and of benefit in the prevention/amelioration of age associated diseases proposedly arising from oxygen radical damage but it has yet to demonstrated that it has any role as a meaningful therapeutic antioxidant. However ascorbate has been promoted by some complementary medicine practitioners for the treatment of cancers but the data in support of an efficacious outcome is equivocal. The rationale for this therapy, has been the antioxidant function of ascorbate to prevent oxidative damage which functions to induce cancer by random oxidation attack on cellular macromolecules. In a series of recent studies by Levine and associates they have revisited the use of high doses of ascorbate as a cancer therapy; their findings are largely summarized in Chen et al. (2005). In one of their earlier studies they reported that increasingly large oral doses of ascorbic acid do not lead to increasing levels of plasma vitamin C; the system is saturable and plasma levels plateau at less than 0.3 mM even on oral doses of 10 g/day. However I.V. doses of 10 g/day result in plasma levels of about 6 mM; doses up to 30–40 g/day have been used. This paper also reports the important discovery that ascorbic acid at a concentration of about 5 mM functions in vitro to selectively kill a variety of cancer cell lines. They demonstrate that under appropriate conditions, specifically, ascorbate at a concentration of about 5 mM in the presence of serum, acts as a prodrug promoting the formation of high concentrations of H_2O_2 which are lethal to a range of human and mouse cancer cell lines. These same high levels of generated H_2O_2 had apparently no effect on the growth of normal human cell lines; the authors had no suggestion as to why H_2O_2 was selective in its action on cancer cell lines. The equivocal earlier findings by others of a beneficial effect of I.V. ascorbate on cancers may possibly be explained by the observation that not all cancer cell lines are killed by ascorbate (Chen et al., 2005) and that there has been a wide variation in reported concentrations of ascorbate administered I.V. However the report of Chen et al. (2005) may be a particularly significant paper; they convincingly argue the case for blinded human clinical trials to be conducted on the possible efficacy of I.V. ascorbate on a range of human cancers.

For the purposes of this review we emphasize that there is no convincing evidence for ascorbic acid acting beneficially in mammals as an antioxidant. On the contrary in large doses it may act as a non-toxic prodrug for the production and delivery of H_2O_2 to tissues, beneficially for the selective treatment of some cancers. Albeit ascorbic acid and coenzyme Q_{10} (and some other antioxidants)

topically applied may have a useful role in the prevention of skin cell damage induced by environmental factors such as over exposure to sun light and UV.

10. The over arching role of coenzyme Q_{10} and hydrogen peroxide in the regulation of the metabolome and the disease process

The over arching normal physiological roles of coenzyme Q_{10} and superoxide anion/H_2O_2 are summarized in Figs. 1a and b and discussed throughout the text.

For over 20 years there have been numerous anecdotal reports of orally administered coenzyme Q_{10} acting beneficially in the treatment of a wide range of apparently unrelated diseases (Table 1). For most practitioners these claims have been greeted with scepticism and coenzyme Q_{10} has not been widely embraced as being therapeutically useful. One exception has been the use of coenzyme Q_{10} for the treatment of mitochondrial disease (MERRF, LHON, MELAS etc.), where clearly mitochondrial energy function is impaired. However, there are the true believers, including the present authors, who consider that the largely anecdotal results most likely have a kernel of truth in them, if only because the reports have come from a diverse range of reputable laboratories. Apart from its obvious role in mitochondrial energy generation to explain the reported clinical benefits, an encompassing physiological antioxidant role has been proposed for coenzyme Q_{10}. There are extant many reports that superoxide/H_2O_2 are toxic products which must be scavenged, otherwise overtime, their formation will result in the development of a plethora of age related diseases. The role of coenzyme Q_{10} (quinol) as an antioxidant has been repeatedly demonstrated in non-physiologically constructed in vitro scenarios, with the observations extrapolated to explain the purported in vivo clinical benefits of coenzyme Q_{10}. However, there are no substantiative clinical findings supporting such a conclusion, just as with the cases of β-carotene, vitamin E, vitamin C and others (Linnane et al., 2007b). In any case this concept appears to be too limited in scope over focus-

Table 1
Reported anecdotal therapeutic benefits with coenzyme Q_{10} on systemic diseases and others

Mitochondrial neuromuscular diseases
Neurodegenerative diseases (Parkinson's disease, dementias, others)
Cognitive function decline, others
Congestive heart failure-prevention/support therapy
Muscle weakness
Chronic fatigue syndrome
Cancer – breast treatment
Chemotherapy amelioration, support for AZT/AIDS treatment
Immuno – regulation
Vascular disease, diabetes mellitus
Sports medicine. Performance enhancement (not a pathology)

Consult Ebadi et al. (2001) and references in the text.
A large literature, comprehensively reviewed in the monographs by Ebadi et al. (2001).

sing on supposed excessive oxidative damage, an hypothesis with which we do not agree.

A more encompassing concept would be that the claimed overarching therapeutic benefits of coenzyme Q_{10} administration (Table 1) revolve around bioenergy generation and H_2O_2 formation. In this scenario coenzyme Q_{10} is concerned with the generation of proton motive force (energy) involving a number of sub-cellular organelles and also as a major regulated source of H_2O_2. It can be readily envisaged that inappropriate up/down-physiological levels of H_2O_2 in specific tissues could lead to a range of systemic disease states which are commonly age associated. A corollary of this concept would be that administrating coenzyme Q_{10} to patients acts therapeutically to restore the H_2O_2 regulated messenger system to its normal physiological level at which it functions to appropriately regulate the metabolome and cell growth/differentiation and resolve the disease problem.

We comment further herein, only on the role of coenzyme Q_{10} in relation to the immune system. Bliznakov and others in a series of publications dating from the 1970's reported on the effect of coenzyme Q_{10} on the immune system responses. Among other phenomena, they reported that coenzyme Q_{10} increased phagocytic activity, humoral antibody response titers, T_4 lymphocyte numbers and decreased immunosuppressive effects of chemotherapeutic drugs (Bliznakov and Hunt, 1986 for overview). There has not been a satisfactory encompassing explanation as to how coenzyme Q_{10} might stimulate immune system activity. We suggest, that this broad based cellular response phenomena, may find an explanation in the coenzyme Q_{10}/H_2O_2 relationship. In this concept H_2O_2 would function by promoting cellular/clonal cell expansion acting in its role as a second messenger for growth; in response to hormones and cytokine effectors responsible for regulation of cell activity and growth.

By way of a concluding comment, it is obvious that no biological system is a perfect machine and that if there is a small inappropriate leakage of free radicals occurring over time, then they may contribute as a factor in the causation of the age associated diseases. Any regulated second messenger system which becomes dysfunctional as a result of loss of up/down regulation will give rise to physiological dysfunction; recall the loss of upward or downward control of any endocrine messenger system and the complex pathologies which result.

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Endogenous synthesis of coenzyme Q in eukaryotes

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Abstract

Coenzyme Q (Q) functions in the mitochondrial respiratory chain and serves as a lipophilic antioxidant. There is increasing interest in the use of Q as a nutritional supplement. Although, the physiological significance of Q is extensively investigated in eukaryotes, ranging from yeast to human, the eukaryotic Q biosynthesis pathway is best characterized in the budding yeast *Saccharomyces cerevisiae*. At least ten genes (*COQ1–COQ10*) have been shown to be required for Q biosynthesis and function in respiration. This review highlights recent knowledge about the endogenous synthesis of Q in eukaryotes, with emphasis on *S. cerevisiae* as a model system.
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1. Overview of coenzyme Q biosynthesis

Cells generally rely on *de novo* synthesis for their supply of Q. Current knowledge about the Q biosynthetic pathway in eukaryotes is mostly derived from characterization of accumulating intermediates in Q-deficient mutant strains of *Saccharomyces cerevisiae*, reviewed by Jonassen and Clarke (2001), Meganathan (1996) and Turunen et al. (2004). Q biosynthesis starts with formation of a hydroxy-benzoic acid head group and a lipophilic polyisoprenoid tail (Olson and Rudney, 1983; Pennock and Threlfall, 1983). The aromatic precursor of the benzoquinone ring is 4-hydroxybenzoic acid (4-HB) derived from tyrosine, an essential amino acid in mammals. In yeast, 4-HB can also be synthesized from chorismate via the shikimate pathway (Goewert, 1980). The building blocks for the synthesis of the polyisoprenyl chain are provided by dimethyl-allyl diphosphate and isoprenyl diphosphate. In yeast and mammals, these five-carbon precursors are derived from

acetyl-coenzyme A via the mevalonate pathway (Grunler et al., 1994).

The putative eukaryotic Q biosynthetic pathway is shown in Fig. 1. First, the polyisoprenoid tail is assembled by polyprenyl diphosphate synthase, which is responsible for determining the number of isoprene units (designated as *n*). Next, polyprenyl diphosphate: 4-HB transferase catalyzes the formation of covalent linkage between the benzoquinone head group and the tail, producing the 4-hydroxy-3-polyprenyl benzoic acid intermediate. The order of subsequent reactions presented in Fig. 1 is speculative, as only a few of the diagnostic intermediates of the blocked steps have been recovered in yeast mutant strains. Proposed modifications of the aromatic ring start with hydroxylation, followed by *O*-methylation, and decarboxylation to form the 6-methoxy-2-polyprenyl phenol intermediate. Afterward, two additional hydroxylations, one *C*-methylation, and one *O*-methylation step are necessary to generate the fully substituted hydroquinone.

So far, nine complementation groups of Q-deficient yeast mutants (*COQ1* through *COQ9*) have been identified (Tzagoloff and Dieckmann, 1990; Johnson et al., 2005). Mammalian homologues of the yeast *COQ* genes have been identified via sequence homology. Human
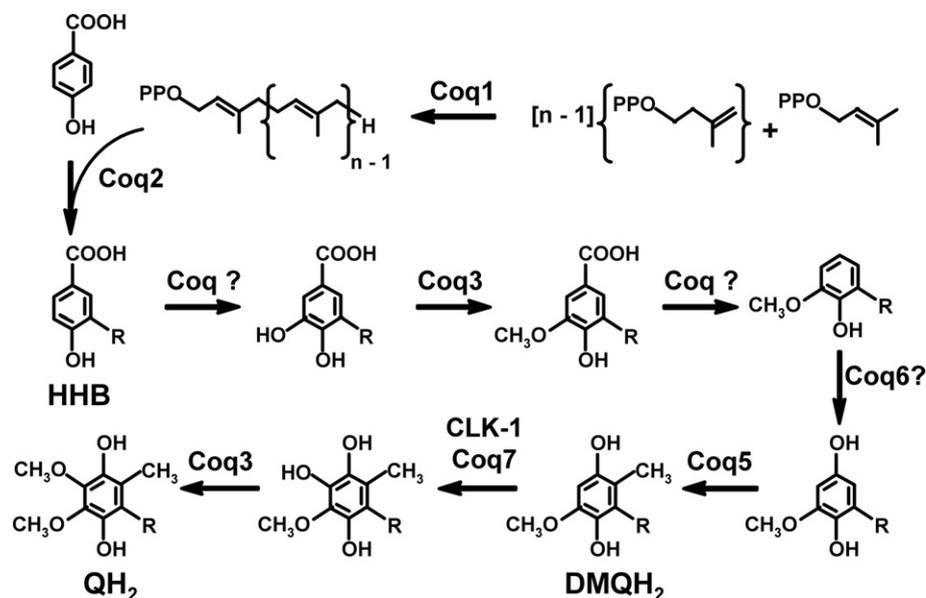

Fig. 1. Proposed Q biosynthetic pathway in eukaryotes. The length of the polyisoprenoid chain of Q, designated by n, varies depending on the species; $n = 6$ in *S. cerevisiae*, 9 in *C. elegans*, and 10 in *H. sapiens*. In *S. cerevisiae*, there are nine identified Coq proteins necessary for the synthesis of QH_2 from dimethylallyl diphosphate and isopentenyl diphosphate precursors. The enzymatic functions of Coq4, Coq6, Coq8, and Coq9 polypeptides have yet to be characterized. Molecular oxygen and AdoMet are proposed donors for the hydroxy and methyl group, respectively (Olson and Rudney, 1983). CLK-1 is the *C. elegans* Coq7 homologue.

homologues of Coq2, Coq3, and Coq7 proteins were demonstrated to functionally complement the corresponding yeast null mutants (Forsgren et al., 2004; Jonassen and Clarke, 2000; Vajo et al., 1999), further indicating that the yeast Q biosynthesis pathway is conserved in humans. The yeast *coq* mutants are non-respiring (unable to grow on non-fermentable carbon sources such as ethanol and glycerol) and petite (forming smaller colonies than wild-type cells when grown on glucose, a fermentable sugar) (Tzagoloff et al., 1975a, 1975b). The hallmark feature of these mutants is that defective NADH–cytochrome *c* reductase and succinate–cytochrome *c* reductase activities in isolated mitochondria of each *coq* mutant strain can be restored to near wild-type level by addition of Q_2 (Tzagoloff et al., 1975b; Johnson et al., 2005). Addition of exogenous Q_6 to *coq* mutants cultured in liquid media with vigorous aeration also restores respiration (Jonassen et al., 1998; Do et al., 2001). Recently, a novel yeast *coq* mutant with defects in respiration and Q-dependent oxidation of NADH and succinate has been identified (Barros et al., 2005). However, unlike the other Q-deficient *coq* mutants (*coq1–coq9*), the *coq10* mutant has nearly normal levels of Q_6, indicating that this protein is not required for Q biosynthesis. Instead, the Coq10 polypeptide may function as a Q-binding chaperone, required for the proper function of Q in respiratory electron transport. The evidence for this proposal is discussed in Section 3.

While Coq1, Coq2, Coq3, Coq5, Coq6, and Coq7 proteins have known or proposed enzymatic functions in Q biosynthesis (Jonassen and Clarke, 2001; Gin et al., 2003) (Fig. 1), it is not clear whether the other Coq proteins also possess enzymatic activities. Coq1 through Coq9

polypeptides localize to the mitochondria (Belogrudov et al., 2001; Gin and Clarke, 2005; Gin et al., 2003; Hsu et al., 1996; Leuenberger et al., 1999; Jonassen et al., 1998; Do et al., 2001; Johnson et al., 2005; Dibrov et al., 1997). *In vitro* mitochondria import were investigated for seven of the yeast Coq polypeptides and demonstrated to be dependent on a mitochondrial membrane potential (Jonassen and Clarke, 2001). Following is a brief discussion about function and submitochondrial localization of the nine Coq proteins, required for Q biosynthesis in eukaryotes (summarized in Table 1). A model incorporating genetic and physical evidence for a yeast Q biosynthetic multi-subunit complex is shown in Fig. 2.

1.1. Coq1

Formation of the *trans*-polyprenyl diphosphate synthase tail in *S. cerevisiae* is catalyzed by the polypeptide encoded by the *COQ1* gene (Ashby and Edwards, 1990), which is responsible for determining the species-specific tail length of Q (Okada et al., 1996). The amino acid sequences of Coq1 protein and related isoprenyl diphosphate synthases from different eukaryotes contain seven highly conserved motifs (Wang and Ohnuma, 2000). Interestingly, expression of Coq1 homologues from a variety of organisms can restore Q biosynthesis and respiration in yeast *coq1* null mutants via production of Q isoforms with distinct number of isoprene units (Okada et al., 1997, 1998). The Coq1 ortholog from the fission yeast *Schizosaccharomyces pombe* (Dps1) fails to complement the *S. cerevisiae coq1* null mutant (Suzuki et al., 1997). However, polyprenyl diphosphate synthases of fission yeast, mouse, and human

Table 1
Characteristics of the nine *S. cerevisiae* Coq proteins required for Q biosynthesis

Yeast protein	Human homolog	Mature M.W.[a] (kDa)	Localized to mitochondria by		Component of Q biosynthetic complex	Complementation of yeast mutants by human homologs
			In vitro import	Submitochondrial fractionation		
Coq1	hDPS1/hDLP1[*]	53	Unknown	Peripheral i.m. matrix side	Unknown	Yes
Coq2	Coq2	30	+	Integral i.m. matrix side	Yes	Yes
Coq3	Coq3	33	+	Peripheral i.m. matrix side	Yes	Yes
Coq4	NP_057119[**]	36	+	Peripheral i.m. matrix side	Yes	Unknown
Coq5	CAI46073[***]	31	+	Peripheral i.m. matrix side	Yes	Unknown
Coq6	NP_872282[***]	51	+	Peripheral i.m. matrix side	Yes	Unknown
Coq7/Cat5	Clk-1/Coq7	23	+	Peripheral i.m. matrix side	Yes	Yes
Coq8/Abc1	Adck-1–Adck5	53	+	Peripheral i.m. matrix side	Unknown	Unknown
Coq9	AAH54340[#]	25	Unknown	Peripheral i.m. matrix side	Yes	Unknown

[a] Via SDS–PAGE migration.
[*] Also known as PDSS1/PDSS2.
[**] NCBI Blastp score > 150.
[***] Blastp score > 200.
[#] Blastp score > 50.
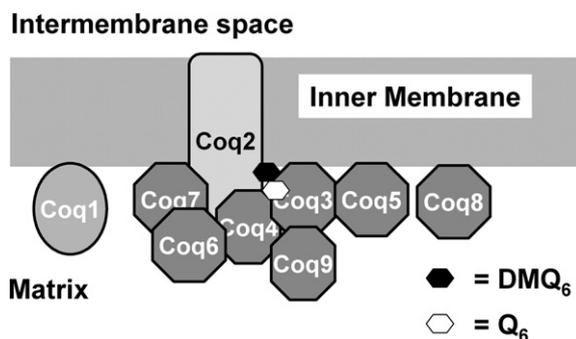
Fig. 2. A model of the mitochondrial Q biosynthetic protein complex in *S. cerevisiae*. The putative complex contains six Coq polypeptides which are peripherally associated with the inner mitochondrial membrane (*dark grey octagons*) and a spanning integral membrane Coq protein (*hatched rectangle*). Proposed lipid components of the multi-subunit complexes include DMQ_6 (*black hexagon*) and the final product Q_6 (*white hexagon*). The stoichiometry of the components has yet to be determined.

are each heterotetramers of two protein subunits, PDSS1 and PDSS2 (Saiki et al., 2003, 2005), while Coq1 from *S. cerevisiae* and the plant *Arabidopsis thaliana* (Jun et al., 2004) function as homo-oligomers. Expression of both subunits of the *trans*-polyprenyl diphosphate synthase of *S. pombe*, mouse or human restores production of the polyisoprene diphosphate and production of Q in complementation assays (Saiki et al., 2003, 2005). Submitochondrial fractionation studies demonstrated that the *S. cerevisiae* Coq1 protein is peripherally associated with the inner mitochondrial membrane on the matrix side (Gin and Clarke, 2005).

1.2. Coq2

The 4-HB polyprenyltransferase is a key enzyme catalyzing the attachment of the polyisoprenoid side chain to the 4-HB ring, generating the first membrane bound Q intermediate, 4-hydroxy-3-polyprenylbenzoic acid. The *S.*

cerevisiae and *Homo sapiens* genes encoding this enzyme are called *COQ2* (Ashby et al., 1992; Forsgren et al., 2004). Ortholog/homologues of Coq2 protein have also been isolated and characterized in other eukaryotes including *S. pombe* (Uchida et al., 2000), *A. thaliana* (Okada et al., 2004), and rice (Ohara et al., 2006). *In vitro* assays in isolated rat liver demonstrated that the polyprenyl diphosphate: 4-HB activity is present mainly in mitochondria (Momose and Rudney, 1972). Polyprenyltransferases involved in Q biosynthesis generally display a lack of specificity for the chain length of the isoprenyl diphosphate substrate (Meganathan, 2001; Gin and Clarke, 2005; Ashby et al., 1992; Okada et al., 2004); however, the specificity was shown to be influenced by Mg^{2+} concentration in whole yeast extracts (Ashby et al., 1992).

Analysis of the predicted amino acid sequence of the *S. cerevisiae* Coq2 protein revealed two conserved putative substrate binding domains found in a family of polyprenyltransferases, six potential membrane spanning domains, and a typical mitochondrial targeting sequence (Ashby et al., 1992). *In vitro* import studies demonstrated that the polypeptide is imported and fully processed within the mitochondria (Leuenberger et al., 1999). Recently, submitochondrial fractionation analysis for the Coq2 protein has been carried out (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation). Coq2 protein behaves as an integral membrane protein associated to the inner mitochondrial membrane, facing the matrix side.

1.3. Coq3

Two *O*-methylation steps in the Q biosynthetic pathway are apparently catalyzed by the same enzyme encoded by *COQ3* gene, as demonstrated by *in vitro* assays with synthetic farnesylated analogs (Poon et al., 1999; Shepherd et al., 1996). The *COQ3* gene was originally identified in *S. cerevisiae* (Clarke et al., 1991) by its ability to restore

Q biosynthesis, and hence respiration, in a *coq3* mutant named C39 (Tzagoloff et al., 1975a; Sippel et al., 1983). Homologues of the *COQ3* gene in rat, *A. thaliana*, and human were subsequently isolated via functional complementation of yeast *coq3* null mutants (Marbois et al., 1994; Avelange-Macherel and Joyard, 1998; Jonassen and Clarke, 2000). The amino acid sequences of the proteins encoded by these *COQ3* homologues all contain four regions that are conserved in a large family of methyltransferase enzymes utilizing *S*-adenosylmethionine (SAM or AdoMet) as the methyl donor (Kagan and Clarke, 1994; Niewmierzycka and Clarke, 1999; Katz et al., 2003) and required a divalent cation (Turunen et al., 2004; Jonassen and Clarke, 2001).

Like most of the other Coq polypeptides, the yeast Coq3 protein also contains a typical mitochondrial targeting sequence at the N-terminus. *In vitro* assays (Hsu et al., 1996) and subcellular localization (Poon et al., 1999) studies showed that the Coq3 preprotein was imported and processed to the mature form in the mitochondria, in a membrane-potential-dependent manner. Further submitochondrial fractionation demonstrated that it is a peripheral protein associated to the matrix side of the inner mitochondrial membrane (Poon et al., 1999). As indicated by the functional complementation and *in vitro* assays mentioned earlier, it is apparent that the Coq3 *O*-methyltransferase has broad substrate specificity. This type of promiscuous substrate recognition is a characteristic shared with catechol-*O*-methyltransferase (COMT), which has numerous physiological substrates including the biosynthetic precursors of dopamine and certain steroids and neurotransmitters (Vidgren et al., 1999). However, the amino acid sequence of COMT fails to show any homology with the yeast Coq3 polypeptide, outside of the conserved methyltransferase motifs (Turunen et al., 2004; Jonassen and Clarke, 2001).

1.4. Coq4

Similar to the Coq3 protein encoding gene, *S. cerevisiae COQ4* gene was cloned via a functional complementation of a Q-deficient *coq4* mutant harboring the E266K point mutation (C9-E1 or *coq4-1*) (Belogrudov et al., 2001). Growth on a non-fermentable carbon source (which requires respiration) caused up-regulation of *COQ4* mRNA steady state levels, consistent with its role in Q biosynthesis. However, the enzymatic function of Coq4 protein, a peripheral protein associated with the inner mitochondrial membrane on the matrix side (Belogrudov et al., 2001) has been a mystery. While it is appealing to speculate that Coq4 protein may serve as a hydroxylase or a carboxylase in the yet-to-be-characterized steps (designated "Coq?" in Fig. 1), the amino acid sequence of Coq4 does not share significant homology with protein domains or motifs with known enzymatic activity. Interestingly, steady-state levels of Coq3 and Coq7 proteins, which are diminished in *coq4* null mutants, are at wild-type levels in the *coq4-1* point mutant (Belogrudov et al., 2001). This result, taken together with recent work demonstrating that the native Coq4 polypeptide co-migrates with Coq3, Coq6, and Coq7 proteins as a high molecular mass complex (Marbois et al., 2005; Tran et al., 2006), indicates that the Coq4 protein has a structural role in the putative polypeptide Q biosynthetic complex (further discussed below).

1.5. Coq5

2-Methoxy-6-polyprenyl-1,4-benzoquinone methyltransferase catalyzes the only *C*-methylation step in the Q biosynthetic pathway, generating the 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinone intermediate. In *S. cerevisiae*, the gene encoding this *C*-methyltransferase is designated *COQ5*. The *COQ5* gene was isolated from a yeast genomic DNA library based on its ability to restore respiratory proficiency in two different *coq5* point mutants, *coq5-1* (Dibrov et al., 1997) and *coq5-2* (Barkovich et al., 1997). Analysis of the *COQ5* promoter region identified consensus binding sites for Gcr1, Mig1, Rtg1/2/3, and Hap2/3/4 transcription factors (Hagerman et al., 2002; Hagerman and Willis, 2002), which regulate gene expression in response to energy sources. Results of Northern blot and Western blot analyses clearly demonstrated *COQ5* expression is up-regulated with glycerol and oleic acid treatments, compared to dextrose, with the highest induction observed during growth on oleic acid (Hagerman et al., 2002; Hagerman and Willis, 2002). The *COQ5* open-reading frame harbors four sequence motifs present in a large family of AdoMet-dependent methyltransferase enzymes (Katz et al., 2003). *In vitro* *C*-methyltransferase assays with the farnesylated analogs of the corresponding intermediates confirmed that Coq5 polypeptide is required for conversion of 2-methoxy-6-polyprenyl-1,4-benzoquinone to 2-methoxy-5-methyl-6-polyprenyl-1,4-benzoquinone in Q biosynthesis (Baba et al., 2004; Barkovich et al., 1997). These enzyme assays further demonstrated that the length of the polyisoprenoid tail does not play a crucial role in substrate recognition of Coq5 protein. Inclusion of NADH is essential for optimal enzymatic activity and is most likely required to convert the quinone to the hydroquinone, generating a nucleophile for the *C*-methyltransfer.

Submitochondrial fractionation analyses demonstrated the Coq5 protein is peripherally associated with the inner mitochondria membrane on the matrix side (Baba et al., 2004). Interestingly, the *coq5-2* and *coq5-5* point mutants maintained normal levels of Coq3, Coq4, and Coq5 polypeptides, while levels of these proteins were greatly diminished in each of the other *coq5* mutants (Baba et al., 2004). These point mutants are the only *coq5* mutants rescued by expression of *Escherichia coli ubiE*, a homolog of *COQ5* (Lee et al., 1997). Taken together, these results indicate that Coq5 protein is essential for the stability and activity of at least two other Coq polypeptides, and provide genetic evidence for a complex of Coq polypeptides in yeast Q biosynthesis.

1.6. Coq6

Functional complementation of a yeast mutant from the G63 (*coq6–1*) complementation group (Tzagoloff and Dieckmann, 1990) resulted in the isolation of the *COQ6* gene (Gin et al., 2003). In contrast to an earlier report (Fiori et al., 2000), *COQ6* is a non-essential gene for viability but is required for growth on non-fermentable carbon sources (Gin et al., 2003). The Coq6 protein is a mitochondrial protein, which is imported in a membrane-potential-dependent manner and peripherally associated with the matrix side of the inner membrane (Gin et al., 2003). *Saccharomyces cerevisiae* Coq6 protein and its homologues in *H. sapiens*, mouse, and *Caenorhabditis elegans* each contains three conserved regions (Gin et al., 2003): an ADP-binding fingerprint (Wierenga et al., 1986), a motif with a putative dual function in FAD/NAD(P)H binding (Eppink et al., 1997), and a consensus sequence that binds to the ribityl moiety of FAD (Eggink et al., 1990). These conserved regions are common features of a large family of FAD-binding-aromatic hydroxylases (Palfey et al., 1995). Consequently, Coq6 protein has been considered as a putative flavin-dependent monooxygenase responsible for adding the hydroxy group to 4-hydroxy-3-polyprenyl benzoic acid and/or 6-methoxy-2-polyprenyl phenol, two uncharacterized hydroxylation steps in Q biosynthesis.

1.7. Coq7

Yeast *COQ7/CAT5* gene was independently isolated and characterized as required for Q synthesis (Marbois and Clarke, 1996; Tzagoloff and Dieckmann, 1990) and involved in carbon catabolite repression/de-repression (Proft et al., 1995). Catabolite repression/de-repression is a global system that regulates transcription of gluconeogenic genes, alternative sugar metabolism, and respiration (Gancedo, 1998). However, the catabolite-regulation defect in *coq7* mutants was later demonstrated to be a secondary effect of respiration deficiency and could be rescued by the addition of exogenous Q_6 (Jonassen et al., 1998), implicating direct involvement of *COQ7* in Q biosynthesis. Moreover, expression of *COQ7* homologues from *C. elegans* (Ewbank et al., 1997), rat (Jonassen et al., 1996), or human (Vajo et al., 1999) were shown to rescue the yeast *coq7* null mutant for growth on non-fermentable carbon sources, indicating functional conservation across species.

Coq7 protein was shown to be required for the hydroxylation of 5-demethoxyubiquinol ($DMQH_2$) (Marbois and Clarke, 1996). Interestingly, G65D *coq7* point mutant was found to accumulate DMQ_6, as well as the earlier intermediate 3-hexaprenyl-4-hydroxybenzoic acid (HHB), though the *coq7* null mutant produced only HHB (Marbois and Clarke, 1996). Similarly, yeast mutants expressing Coq7 protein with the missense mutation (E194K) produced DMQ_6, while DMQ_6 was not detected in strains harboring a *coq7*-nonsense mutation, where the carboxyl-terminal half of the protein is missing (Padilla et al., 2004). These results suggest that Coq7 protein is either involved in one or more mono-oxygenase steps or serves as an essential component of the putative multi-subunit enzyme complex. Biochemical function of Coq7 protein as a hydroxylase was further supported by the determination that it belongs to a family of di-iron binding oxidases containing a conserved sequence motif for the iron ligands, EXXH (Stenmark et al., 2001). Coq7 homologues from *Pseudomonas aeruginosa*, *Thiobacillus ferroxidans*, *C. elegans* restored Q biosynthesis in an *Escherichia coli ubiF* mutant (Adachi et al., 2003; Stenmark et al., 2001). *E. coli* UbiF, a flavin-dependent DMQ hydroxylase that shares no homology to Coq7 protein, has been shown to functionally complement both the *coq7* null mutant and the DMQ_6-producing *coq7E194K* mutant, with better efficiency in the latter (Tran et al., 2006). Collectively, these findings indicate that yeast Coq7 protein functions in the hydroxylation of DMQ. Moreover, steady state levels of the Coq3, Coq4, and Coq6 polypeptides were higher in the *coq7E194K* mutant than in the null mutant, suggesting that Coq7 protein and DMQ_6 serve to stabilize other Coq polypeptides.

Recent submitochondria fractionation studies (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation) demonstrate that yeast Coq7 protein, like its homologues in mice (Jiang et al., 2001), is peripherally associated to the inner membrane on the matrix side. However, earlier studies have modeled Coq7 as an interfacial inner mitochondrial membrane protein (Stenmark et al., 2001; Berthold and Stenmark, 2003). Interfacial membrane proteins, such as prostaglandin synthase (Picot et al., 1994) and squalene cyclase (Wendt et al., 1999), are embedded in the membrane via interaction with only one leaflet of the bilayer. Unlike the Coq7 polypeptide, proteins classified as interfacial (based on X-ray crystal structures), including prostaglandin synthase, squalene cyclase, fatty acid amide hydrolase, and microsomal cytochrome P450, each behaved as integral proteins in biochemical assays (Bracey et al., 2004). The true nature of the Coq7 protein-membrane association awaits a structure determination for yeast Coq7p or one of its homologues.

1.8. Coq8

The *COQ8* gene was initially identified as *ABC1* (activity of bc_1 complex) for its ability to partially suppress, in multi-copy, the cytochrome *b* translation defect due to the *cbs2–223* mutation in the *CBS2* gene (Bousquet et al., 1991). *CBS2* is a yeast nuclear gene encoding a translational activator of cytochrome *b* (Rodel, 1986). It was observed that inactivation of *ABC1* resulted in respiratory defect and absence of NADH–cytochrome *c* reductase activity (Bousquet et al., 1991); a phenotype similar to that of Q-deficient strains (Tzagoloff and Dieckmann, 1990). It was subsequently shown that the respiratory

complexes II, III, and IV of the *abc1* null mutant were thermo-sensitive and addition of exogenous Q could partially compensate for the respiratory deficiency (Brasseur et al., 1997). These results led to a hypothesis that the *ABC1* gene product may function as a chaperone that is essential for the proper conformation and activity of the *bc₁* and its neighboring complexes (Brasseur et al., 1997). However, Do et al. (2001) demonstrated that the *COQ8* gene, required for Q biosynthesis (Poon et al., 1997), is the same as the *ABC1* gene and provided data indicating that Q deficiency is exclusively responsible for the pleiotropic defects of *abc1/coq8* mutants. Moreover, a neighboring tRNA^{TRP} gene located downstream of *COQ8/ABC1* gene was demonstrated to account for the suppression of the *cbs2-223*, a UGA nonsense mutation (Hsieh et al., 2004). Although, its biochemical function in Q biosynthesis is currently unknown, Coq8/Abc1 protein has been classified as putative protein kinase based on the identification of four kinase conserved motifs in its amino acid sequence (Leonard et al., 1998).

1.9. Coq9

The *COQ9* gene was recently identified and characterized as a new gene that, when mutated, results in a Q-deficient phenotype, in a similar manner to other *COQ* genes (Johnson et al., 2005). However, the function of Coq9 protein in Q biosynthesis is not yet known. The amino acid sequence of Coq9 protein contains a distinct conserved domain present in the COG5590 protein family (Marchler-Bauer et al., 2005). While *COQ9* homologues are well-represented in genomes of eukaryotes and α-proteobacteria, Coq9 protein has no homology to proteins with known function. Intriguingly, multi-copy expression of the *COQ8* gene was shown to restore respiration in a specific *coq9* point mutant (*coq9-1*; E151STOP nonsense mutation). Although a small amount of Coq9 polypeptide was detected in the *coq9-1* nonsense mutant strain, levels were not elevated by the multi-copy suppression mediated by the *COQ8* gene (Johnson et al., 2005; Hsieh et al., 2007). Consequently, the mechanism responsible for the multi-copy *COQ8* suppression of *coq9-1* is unknown.

Based on the mobility in the SDS–PAGE, the molecular mass of Coq9 protein is about 25 kDa (Hsieh et al., 2007), slightly smaller than the predicted precursor (29.9 kDa) (Johnson et al., 2005), and is consistent with the removal of a putative mitochondrial targeting sequence. However, the native size of Coq9 protein estimated from its sedimentation on sucrose gradients is about three times larger, indicating that the protein is either a homo-oligomer or in a complex with other proteins (Johnson et al., 2005). Potential partners in such a complex are Coq3 and Coq5 polypeptides, which were shown to co-sediment with the Coq9 protein. Recently, submitochondrial localization analysis has demonstrated that Coq9 protein is a peripheral membrane protein associated with the matrix side of the mitochondrial inner membrane (Hsieh et al., 2007).

2. *Saccharomyces cerevisiae* Q biosynthesis requires a multiple-enzyme complex or complexes

There are many well-characterized mitochondrial respiratory protein complexes in yeast, for example, cytochrome oxidase, ATP synthase, and the cytochrome *bc₁* complexes. In these systems, the absence or mutation in one component results in proteolytic degradation, instability, or inactivation of the remaining subunits (Glerum et al., 1997; Tzagoloff et al., 1994). Multi-subunit enzyme complexes allow channeling of labile/reactive intermediates, enhance catalytic efficiency, and provide a mechanism for coordinative regulation of components. This seems to be the case in Q biosynthesis as well.

Previous studies have provided numerous lines of genetic evidence for a Q biosynthetic complex and for interdependent relationship among Coq polypeptides. Each of the null *coq3* to *coq9* mutants predominantly accumulates the same earlier intermediate HHB, the product of Coq2p, instead of the corresponding diagnostic intermediate (Poon et al., 1995, 1997; Johnson et al., 2005). Steady state levels of Coq3, Coq4, Coq6, Coq7, and Coq9 polypeptides are significantly decreased in mitochondria isolated from any of the other *coq* null mutants (Hsu et al., 2000; Baba et al., 2004; Belogrudov et al., 2001; Gin and Clarke, 2005; Tran et al., 2006; Hsieh et al., 2007). In addition, a null mutation in any of the *COQ* genes led to decreased Coq3 *O*-methyltransferase activity, although *COQ3* RNA levels were not affected (Hsu et al., 2000). These phenotypes were not due to a lack of respiration, because other mutants with defects in the respiratory complexes such as *atp2* and *cor1* null mutants retained wild-type levels of *O*-methyltransferase activity. Although Coq1 polypeptide levels remain unchanged in any of the other *coq* null mutants, the protein itself and/or its lipid product appears to be essential for stabilization of Coq3, Coq4, Coq6, Coq7, and Coq9 proteins (Gin and Clarke, 2005; Hsieh et al., 2007). It has been demonstrated that phenotypes of certain *coq* point mutants dramatically differ from the respective null mutants. For example, the *coq7E194K* point mutant but not the *coq7* null mutant was rescued by low copy expression of *E. coli ubiF* (Tran et al., 2006). Similarly, the *coq5-2* and *coq5-5* mutants, which have normal levels of Coq3, Coq4, and Coq5 polypeptides, are the only two *coq5* mutants significantly rescued by expression of *E. coli ubiE*, a homolog of *COQ5* gene (Baba et al., 2004). Moreover, the *coq4–1* (E226K) mutant maintained wild-type levels of Coq3 and Coq7 polypeptides, which were greatly diminished in the null *coq4* mutants (Belogrudov et al., 2001). This data renders support for the proposed structural/regulatory role of Coq4 protein in a multi-protein complex in Q biosynthesis. In such a model, the complete absence of Coq4p results in instability of several of the other Coq polypeptides, while certain amino acid substitution mutations in the Coq4 protein serve to stabilize the Coq polypeptides. Taken together, these results are

consistent with the involvement of the Coq polypeptides and/or the Q intermediates formed by these proteins in a multi-subunit complex or complexes. Postulated lipid components of the Q biosynthetic complex may also include the final product Q_6 because the addition of exogenous Q was shown to stabilize steady-state levels of Coq3 and Coq4 polypeptides in the null *coq7* mutant (Tran et al., 2006).

Recent biochemical analyses provide physical evidence for the model of Q biosynthetic complex. Size exclusion chromatography (gel filtration) coupled with *O*-methyltransferase assays of the supernatant from digitonin-solubilized mitochondria demonstrated that Coq3, Coq4, Coq6, and Coq7 polypeptides co-elute as a high molecular mass complex with the Coq3 *O*-methyltransferase activity (Marbois et al., 2005; Tran et al., 2006). Further analysis of the representative gel-filtration fractions with mass spectrometry indicated that the DMQ_6 intermediate, the substrate of Coq7 protein, is also associated with the complex (Marbois et al., 2005). Recent gel filtration analysis using newly generated specific antibodies showed that Coq2 protein co-elutes with Coq4 and Coq7 polypeptides (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation), and Coq9 polypeptide co-elutes with Coq3, Coq4, Coq5, and Coq8 proteins (Hsieh, E. J. and Clarke, C. F., unpublished data). Additionally, two-dimensional Blue native gel electrophoresis (BN–PAGE/SDS–PAGE) analyses of the supernatant from digitonin-solubilized mitochondria yield data indicating that Coq2, Coq3, Coq4, Coq7, and Coq9 polypeptides co-migrate as a high molecular mass complex or complexes (Tran et al., 2006) (Tran, U.C., Gulmezian, M., Santos-Ocaña, C., Saiki, R., Navas, P., and Clarke, C. F., manuscript in preparation) (Hsieh et al., 2007). Co-precipitation of biotinylated-Coq3 protein with Coq4 identified a physical interaction between Coq3 and Coq4 polypeptides (Marbois et al., 2005). Moreover, Coq9-HA (hemagglutinin antigen) fusion protein was recently demonstrated to physically interact with Coq4, Coq5, Coq6, and Coq7 polypeptide via co-precipitation. All together, these results support the existence of a multi-subunit-Q biosynthetic complex or complexes consisting of the Coq polypeptides and some of the corresponding lipid Q intermediates.

A proposed model for the Q biosynthetic complex in which Coq2 serves as an anchor to the inner mitochondrial membrane is depicted in Fig. 2. In this figure, peripheral protein components of the complex are modeled in association with Coq2. However, the complex could also be anchored to the membrane via other uncharacterized polypeptides and/or lipid components of the inner membrane. Further studies are needed to determine the stoichiometry of the polypeptide and lipid components of the complex, elucidate the nature of the membrane association, identify other potential constituents, and examine the effects of *coq* mutations on the dynamic of the complex.

3. Regulation of Q function and biosynthesis in *S. cerevisiae*

3.1. A potential Q_6 binding polypeptide-Coq10 protein

Complementation of a partially respiratory deficient mutant from the Genome Deletion Strain Collection identified *COQ10*, a new gene required for Q function in respiration (Barros et al., 2005). Similar to previously characterized *coq* mutants (*coq1–coq9*), the yeast *coq10* mutant exhibits defective NADH–cytochrome *c* reductase and succinate–cytochrome *c* reductase activities, which can be restored to near wild-type level by addition of Q_2. Unlike the other *coq* mutants, however, the *coq10* mutant grows slowly on medium containing non-fermentable carbon sources (ethanol and glycerol) and produces near wild-type levels of Q_6. Interestingly, the slow-growing phenotype on of the *coq10* mutant on medium containing ethanol and glycerol was partially rescued by exogenous Q_6 supplementation or multi-copy expression of the *COQ2*, *COQ7*, or *COQ8* genes. This suggests that endogenous Q_6 produced by the mutant is not as "functional" as that synthesized in the corresponding wild-type strain. Sequence analysis of yeast Coq10 protein, as well as its homologues in *Caulobacter crescentus* and other eukaryotes, identifies it as a member of the protein superfamily containing the START domain (Shen et al., 2005). The solution structure of the *C. crescentus* homolog of Coq10 identified a hydrophobic tunnel which in other START family members functions in binding cholesterol, polyketides, or phospholipids (Miller, 2007). Because polypeptides belonging to this superfamily have been shown to be involved in lipid binding and trafficking, it is likely that Coq10 protein may function in transport and/or directing newly synthesized Q to its correct location in the mitochondrial electron transport chain.

3.2. Effects of carbon catabolites on Q biosynthesis

In *S. cerevisiae*, Q levels are directly correlated to mitochondrial development and oxygen availability (Pennock and Threlfall, 1983). Biosynthesis of Q was found to be highest in aerobically grown cells and barely detectable in anaerobic cells (Lester and Crane, 1959). When yeasts were grown in aerobic batch cultures, the amount of Q varied depending on carbon catabolites (Gordon and Stewart, 1969). High glucose concentration inhibited Q biosynthesis to a higher degree than similar concentration of galactose, a non-repressing fermentable carbon source. As expected, Q production was greatly increased in media containing non-fermentable carbon source, when catabolite repression is at the minimum. Interestingly, supplementation with cAMP alleviates the inhibitory effect of glucose on Q biosynthesis at the enzymatic level (Sippel et al., 1983). Previous studies have demonstrated that mRNA levels of *COQ3*, *COQ4*, *COQ5*, and *COQ7* genes were higher in yeasts grown in glycerol containing media than in cultures containing fermentable dextrose (Marbois and Clarke, 1996; Hagerman et al., 2002; Clarke et al.,

1991; Belogrudov et al., 2001). The amount of Coq7 polypeptide was significantly increased by growth on media containing ethanol (Jonassen et al., 1998). To further understand the mechanism that underlies this carbon-catabolite regulatory control of Q biosynthesis, it is necessary to examine how growth in media containing different carbon sources affects Q_6 content, steady state levels of Coq proteins, and the dynamic of the multi-subunit-Q biosynthetic complex.

4. Perspectives

Coenzyme Q (ubiquinone or Q) is a prenylated benzoquinone lipid that is found in membranes throughout eukaryotic cells. The reversible redox chemistry of Q is responsible for its function in the respiratory electron transport chain of inner mitochondrial membranes and as a lipophilic antioxidant. Q is widely used as a dietary supplement and in a variety of clinical therapies, including treatment of several neuro-degenerative diseases (Ferrante et al., 2002; Grundman et al., 2002; Muller et al., 2003; Beal, 2004; Shults, 2005) and certain respiratory chain defects (Geromel et al., 2002).The studies reviewed in this article employed a combination of genetics, molecular biology, and biochemistry to delinate the eukaryotic biosynthetic pathway of Q, with *S. cerevisiae* as model system. Considering the nutritional and therapeutic aspects of Q, it is likely that characterization of Q biosynthesis and regulation will promote our understanding Q metabolism and its recent use in clinical therapies.

Acknowledgements

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Review

The uptake and distribution of coenzyme Q(10)

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Abstract

This review describes recent advances in our understanding of the uptake and distribution of coenzyme Q10 (CoQ10) in cells, animals, and humans. These advances have provided evidence of important pharmacokinetic factors, such as non-linear absorption and enterohepatic recirculation, and may facilitate the development of new CoQ10 formulations. Studies providing data which support the claim of tissue uptake of exogenous CoQ10 are also discussed. Improved CoQ10 dosing and drug level monitoring guidelines are suggested for adult and pediatric patient populations. Future CoQ10 research should consider uptake and distribution factors to determine cost-benefit relationships.
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Keywords: Coenzyme Q(10); Ubiquinone; Absorption; Uptake; Distribution; Pharmacokinetics

1. Introduction and objectives

For the past 10 years the frequency of studies involving coenzyme Q10 (CoQ10) (also known as ubiquinone-10) has increased in both basic and clinical research areas (Littarru and Tiano, 2005). As a result we now have an improved understanding of the pharmacokinetics and pharmacodynamics of CoQ10. Clinical studies have demonstrated the importance of dose-ranging trials and monitoring plasma levels of CoQ10 (Shults et al., 1998, 2002, 2004; Kaikkonen et al., 2002; Ferrante et al., 2005; Miles et al., 2006). In addition, interest in CoQ10 has grown because of evidence that exogenous CoQ10 supplementation may support and stabilize mitochondrial oxidative phosphorylation (Santos-Ocana et al., 2002; Marriage et al., 2004; Geng and Guo, 2005; Yen et al., 2005); function as a gene regulator (Linnane et al., 2002; Groneberg et al., 2005); and provide protective benefits for certain cell types (Matthews et al., 1998; Yen et al., 2005; Somayajulu et al., 2005; Klongpanichapak et al., 2006; Kooncumchoo et al., 2006; Sharma et al., 2006).

While a few studies have suggested CoQ10 may provide therapeutic benefits in mitochondrial diseases, a recent scientific review of all treatments concluded that there is no clear objective evidence supporting the use of any intervention in mitochondrial disorders (Chinnery et al., 2006). We now know that many CoQ10 studies were hampered by dosing- and monitoring-related factors. Examples of inadequate CoQ10 dosing are common in the medical literature (Bresolin et al., 1990; Permanetter et al., 1992; Hofman-Bang et al., 1995; Strijks et al., 1997; Costeff et al., 1998; Henriksen et al., 1999; Khatta et al., 2000). Failure to monitor CoQ10 plasma levels made it difficult to verify absorption and medication compliance in some studies (Permanetter et al., 1992; Strijks et al., 1997; Huntington Study Group, 2001). Also the significance of the inter-individual variability in CoQ10 absorption may have not been appreciated by others (Kaikkonen et al., 2002). The importance of dosage and monitoring considerations in CoQ10 research cannot be overemphasized.

The objectives of this review are threefold: (1) to review pertinent studies involving CoQ10 absorption and distribu-

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tion pharmacokinetics (PK); (2) to summarize evidence supporting CoQ10 uptake and distribution at a cellular level; and (3) to propose clinical guidelines for dosing and monitoring CoQ10 therapy.

2. Coenzyme Q10 pharmacokinetics

2.1. Gastrointestinal uptake considerations

Biochemical characteristics of CoQ10 are important for our understanding of uptake and distribution following oral ingestion. CoQ10 is practically insoluble in aqueous solutions because of its lipophilic 10-carbon chain. Because of its lipid characteristics, it appears that the first step in the uptake of exogenous CoQ10 is incorporation into chylomicrons for transport into the lymph and to the peripheral blood. Like other lipids CoQ10 absorption is probably a complex process and dependent upon active transport mechanisms.

An intravenous form of CoQ10 is not currently available for human use, so the absolute bioavailability of CoQ10 cannot be measured. Still it is useful to consider the time course profile of CoQ10 following oral administration. PK studies have shown that after oral administration

CoQ10 plasma levels begin to increase above baseline levels within 1–2 h, if taken on an empty stomach (Fig. 1a) (Tomono et al., 1986; Weis et al., 1994; Miles et al., 2002; Hosoe et al., 2007). The time (T_{max}) of peak plasma concentration (C_{max}), which generally occurs ~6–8 h following a dose, may be less predictable with non-solubilized formulations compared to solubilized forms (Fig. 1a) (Tomono et al., 1986; Weis et al., 1994; Miles et al., 2002; Hosoe et al., 2007).

The site of uptake of exogenous CoQ10 from the small intestine has not been clearly described in humans, but was recently evaluated in an isolated rat gastrointestinal model (Palamakula et al., 2005). The highest CoQ10 permeability of the model was associated with the duodenum, followed by colon > ileum > jejunum (Palamakula et al., 2005). The authors suggest that CoQ10 absorption appears to be a complex process, which may involve both active and passive mechanisms (Palamakula et al., 2005). The permeability of the ileum and colon regions also supports the possibility of enterohepatic recirculation of CoQ10.

A non-linear or zero-order absorption process in the gastrointestinal tract is often associated with an active transport process. PK modeling of deuterated CoQ10 absorption was best described as zero-order in 16 healthy volunteers (Tomono et al., 1986). Evidence of non-linear CoQ10 absorption was also suggested by dose ranging studies, where CoQ10 plasma concentrations are proportionately less as dosage is increased. After 28 days of ubiquinol-10 (the reduced form of CoQ10) intake by 20 healthy volunteers, a 90 mg/day dose attained a mean plasma CoQ10 concentration 2.27 μg/mL above baseline concentration (Hosoe et al., 2007). A 300 mg/day dose over the same period achieved a mean plasma CoQ10 concentration of 6.62 μg/mL above baseline (Hosoe et al., 2007). With linear or first-order absorption, the 300 mg dose would have been predicted to plateau at ~7.56 μg/mL above baseline (Hosoe et al., 2007). In a double-blind, randomized, placebo-controlled trial of an all-*trans* form CoQ10 Ikematsu et al. showed that after 4 weeks plasma CoQ10 levels increased only slightly higher (or ~3.0 μg/mL above baseline) in 22 healthy adults taking 900 mg/day compared with 21 individuals taking 300 mg/day (or ~2.0 μg/mL above baseline) (Ikematsu et al., 2006). Interestingly, the results of these two studies provide evidence that the CoQ10 biochemical form may also affect bioavailability. Specifically, the increase above baseline with the 300 mg/day dosage of ubiquinol-10 (Hosoe et al., 2007) was >3-fold more than the same dosage of all-trans form of CoQ10 (Ikematsu et al., 2006).

Further evidence of non-linear CoQ10 absorption has been provided by three patient studies. Shults et al. reported that a fourfold increase in CoQ10 dosage from 300 to 1200 mg/day resulted in only approximately a 2.5-fold increase in mean plasma concentration, i.e. from ~1.4 to ~3.4 μg/mL (Shults et al., 2004). Two other studies found no significant difference in mean plasma CoQ10 concentrations between 2400 and 3000 mg/day dosages (Shults
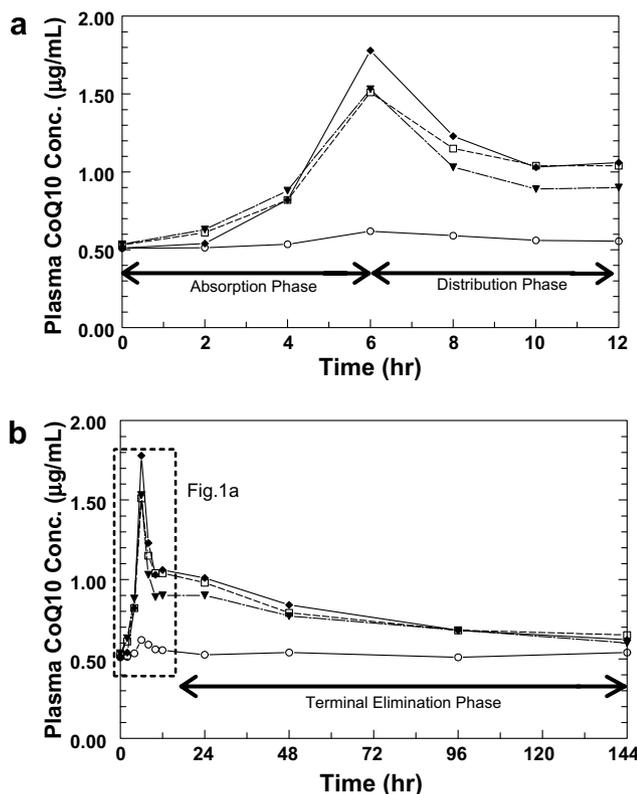
Fig. 1. Comparison of the plasma concentration curves over the first 12 h (a) and over 144 h (b) following single 180 mg doses of four coenzyme Q10 products in adult volunteers. Products A (-□-), B (-◆-), and C (-▼-) contained solubilized coenzyme Q10, and product D (–) contained non-solubilized coenzyme Q10 powder (Miles et al., 2002). Reproduction permission obtained from Elsevier.

et al., 2004; Ferrante et al., 2005). These results suggest that the absorption of CoQ10 may be limited in some individuals. Dosages which exceed the absorptive capacity for CoQ10 may have minimal effect on efficacy, and unnecessarily increase the cost of treatment.

It should also be noted that because of the complexity of CoQ10 absorption, its bioavailability may also be affected by other factors. Some have suggested that the effect of food on CoQ10 absorption PK may be significant, but few data are available to support this postulate. Comparison of 30 mg doses of CoQ10 in diet and capsule found not difference in C_{max} (Weber et al., 1997), however this study did not directly measure the effect of food on CoQ10 bioavailability. Further studies are needed to clarify whether age, gender, lipoprotein status, diet, dosage formulation, or other factors may affect the bioavailability of CoQ10 with chronic dosing.

As suggested previously CoQ10 bioavailability appears to be ''formulation-dependent''. Inter-product variability appears to be several-fold according to single-dose (Kommuru et al., 2001; Miles et al., 2002) and multiple-dose (Chopra et al., 1998; Molyneux et al., 2004) PK studies. Recent in vitro data suggest that uptake of CoQ10 by Caco-2 cells may be enhanced by solubilization (Bhagavan et al., 2007). Inter-individual variability in CoQ10 absorption, indicated by coefficients of variation from 30% to 50% for areas of the concentration–time curve, may also be an important factor (Tomono et al., 1986; Weis et al., 1994; Chopra et al., 1998; Miles et al., 2002). These findings underscore the need for monitoring CoQ10 plasma levels during clinical studies, particularly when untested CoQ10 formulations are administered.

2.2. Distribution PK considerations

The overall concentration–time curve for CoQ10 has been shown to best fit a 3-compartment PK model (Tomono et al., 1986). After gastric emptying CoQ10 is thought to be absorbed along with other lipids as chylomicron particles in the small intestine, and then taken up by liver cells (Scalori et al., 1990; Reahal and Wrigglesworth, 1992). The distribution phase following the C_{max} occurs during the 6–12 h period after the C_{max} (Fig. 1a).

In the liver CoQ10 is incorporated with lipoproteins and released into the blood. In plasma CoQ10 is almost totally associated with lipoproteins, where it has greatest affinity for VLDL and LDL-cholesterol (Elmberger et al., 1989; Tomasetti et al., 1999; Kaikkonen et al., 2002). Interestingly, a secondary plasma peak has also been described ~24 h following CoQ10 administration (Fig. 1b) (Tomono et al., 1986; Weis et al., 1994; Miles et al., 2002). The secondary peak is most likely a result of enterohepatic recirculation, where CoQ10 is released along with bile via the biliary tract and partially re-absorbed during a second pass through the small intestine. Enterohepatic recirculation may contribute to the wide variation in bioequivalence of drugs which undergo this process (Horkovics-Kovats,

1999). Further studies are needed to clarify the importance of enterohepatic recirculation on CoQ10 bioavailability, however.

There is general agreement regarding the importance of lipoproteins as transporters of CoQ10 in plasma. However, there are questions regarding the dependency between CoQ10 and hepatic lipoprotein biosynthesis (Pronzato et al., 1988; Wojcik et al., 2003). Infusion of bile acid blocking agents in sheep significantly decreased bile acid content, but did not affect CoQ10 concentrations in bile (Wojcik et al., 2003). These results seem to suggest that CoQ10 secretion from the liver may be independent of hepatic cholesterol biosynthesis. More studies are needed to evaluate the effect of exogenous CoQ10 supplementation on hepatic secretory function, and the role of enterohepatic recirculation in CoQ10 bioavailability.

3. Cellular uptake considerations

3.1. Animal studies

Some have questioned whether exogenously administered CoQ10 is taken up by peripheral cells and tissues. Early studies in rat (Scalori et al., 1990; Reahal and Wrigglesworth, 1992; Zhang et al., 1995; Lönnrot et al., 1998) and mouse (Lass et al., 1999), at doses ranging from 10 to 123 mg/kg/day, indicated that CoQ10 tissue uptake was limited mainly to the liver and plasma. However, one of the earliest and most notable reports studied the systemic distribution and uptake of [14C]-CoQ10 following intravenous dose (0.6 mg/kg) administration to guinea pigs (Yuzuriha et al., 1983). The guinea pig model showed that after the dose [14C]-CoQ10 was taken up from blood by peripheral tissues for as long as 24 h, and that concentrations in heart, adrenal, and brain tissues peaked at approximately 24 h (Yuzuriha et al., 1983). The fact that CoQ9 is the predominant endogenous form of CoQ in rats and mice, in contrast to CoQ10 in the guinea pig, may explain the differences between the findings of these studies.

It should be noted that more recent animal studies (Kwong et al., 2002; Kamzalov et al., 2003), which utilized extremely high doses of CoQ10 ranging from ~150 to 650 mg/kg/day, reported increased CoQ10 content in tissues and mitochondrial of rats and mice. This dosage range is probably unacceptably high for human purposes, so their results may not be extrapolated to humans. However, advances in pharmaceutical technology should encourage the development of new CoQ10 formulations which may facilitate the tissue uptake of CoQ10 (Ratnam et al., 2006).

3.2. Human studies

The 3-compartment PK model described previously (Tomono et al., 1986), suggests that after gastrointestinal uptake and release from the liver, CoQ10 is distributed to peripheral tissues separate from the central compartment.

This complex PK model may also explain the prolonged terminal elimination phase as CoQ10 is redistributed from peripheral tissues into plasma. In healthy volunteers a prolonged terminal elimination half-life of CoQ10, i.e. ~33 h, was reported following a single dose of deuterated CoQ10 (Tomono et al., 1986). Similar results have been reported other studies, where ~5 to 6 days were required for plasma CoQ10 levels to return to baseline following a single dose (Fig. 1b) (Weis et al., 1994; Miles et al., 2002). A multiple-dose study reported that 2 weeks after discontinuation of a 4-week CoQ10 treatment period at 90 mg/day and 300 mg/day, plasma levels were still 30% and 74%, respectively, higher than baseline levels (Hosoe et al., 2007). Further proof of CoQ10 uptake and slow redistribution was provided in the guinea pig model, where [14C]-CoQ10 activity in adrenal, heart, kidney, and brain tissues was detected for over 7 days after a single intravenous dose (Yuzuriha et al., 1983). These data from human and animal studies provide good evidence that exogenous CoQ10 may be taken up by peripheral tissues, then slowly redistributes into plasma for at least 2 weeks depending upon the length of the dosing period. This is an important consideration for designing "washout" periods between treatments in clinical studies.

There is increasing evidence that the cellular uptake of CoQ10 may influence certain aspects of subcellular function. The effects of exogenous CoQ10 on mitochondria have been studied in several cell models, including skeletal muscle (Linnane et al., 2002), lymphocyte (Marriage et al., 2004), platelet (Niklowitz et al., 2004), neuronal (Somayajulu et al., 2005), CaCo-2 (Groneberg et al., 2005), and HL-6 (Fernandez-Ayala et al., 2005a,b) cells. Fernandez-Ayala et al. compared the effects of coenzyme Q6 (CoQ6) and CoQ10 supplementation on human HL-6 cells (Fernandez-Ayala et al., 2005a). They found increased mitochondrial CoQ content with exposure to both cofactors. However, only CoQ10 maintained mitochondrial respiration. CoQ6 impaired mitochondrial function, and transiently decreased CoQ10 content in this cell model (Fernandez-Ayala et al., 2005a). Another report found increased ATP synthesis in isolated lymphocytes from patients with oxidative phosphorylation disorders, who were receiving CoQ10 treatment (Marriage et al., 2004). It is also notable that in control lymphocytes they also found a dose-dependent relationship between CoQ10 and ATP synthesis (Marriage et al., 2004). Their study found no significant effect on ATP synthesis when lymphocytes were incubated other vitamins and cofactors, including ascorbic acid, vitamin E, riboflavin, thiamine, niacin, vitamin K, and carnitine (Marriage et al., 2004). The authors suggest that CoQ10 may have a beneficial effect in the treatment of oxidative phosphorylation disorders (Marriage et al., 2004). These reports support the claim that exogenous CoQ10 may be transported to and taken up by peripheral cells. However, further investigations are needed for confirmation of these effects.

4. Clinical guidelines and future research

Evidence of a dose–response relationship with CoQ10 supplementation was suggested by Shults et al. (2002), and provided a basis for establishing CoQ10 dosage and monitoring guidelines (Shults et al., 2004; Steele et al., 2004; Ferrante et al., 2005; Miles et al., 2006). Studies, which considered CoQ10 gastrointestinal uptake and distribution factors, have contributed to the recognition of the excellent safety profile and dosage guidelines for CoQ10 in adult (Shults et al., 2004) and pediatric (Miles et al., 2006) patient populations. Future dose-ranging trials may help answer the question whether an optimal therapeutic or target range exists for CoQ10 in plasma.

CoQ10 dosage guidelines, which appeared to be safe and well tolerated, were recently suggested for adults (up to 1200 mg/day) (Hathcock and Shao, 2006) and for children (up to 10 mg/kg/day) (Miles et al., 2006). Monitoring trough CoQ10 plasma concentrations may be considered after 3–4 weeks of constant dosing, when steady-state conditions exist (Chopra et al., 1998; Hosoe et al., 2007). Steady-state plasma concentrations at these dosage levels generally ranged from 5 to 10 μg/mL (Shults et al., 2002, 2004; Ferrante et al., 2005; Miles et al., 2006). This range is 2.5- to 5-fold higher than the upper limit of the 95% CoQ10 reference intervals for healthy adults (Steele et al., 2004). This author would suggest that achieving CoQ10 plasma levels in a target range from 5 to 10 μg/mL may be useful to verify dosing adequacy and to assess medication compliance. However, this target range should not be considered as a requirement for optimizing CoQ10 dosage.

Important questions involving the uptake and distribution of CoQ10 in the treatment of human diseases still remain unanswered. For example, how do PK-related factors such as non-linear absorption affect cost-benefit relationships? Do uptake and distribution factors affect interactions between CoQ10 and other drugs, e.g. vitamin E (Kaikkonen et al., 2002; Sohal, 2004), corticosteroids (Gvozdjakova et al., 2005), HMG-CoQ reductase inhibitors (statins) (Hargreaves et al., 2005), warfarin (Zhou et al., 2005), green tea (Upaganlawar et al., 2006), and angiotensin-converting enzyme (ACE) inhibitors (Sander et al., 2006)? Does exogenously administered CoQ10 distribute into peripheral body fluids and tissues, e.g. semen (Balercia et al., 2004), saliva (Sekine et al., 2005), epidermis (Ashida et al., 2005), placental tissues (Teran et al., 2005), breast milk (Niklowitz et al., 2005), skeletal muscle (Rosenfeldt et al., 2005; Horvath et al., 2006), and cerebrospinal fluid (Isobe et al., 2006), and thereby affect disease processes or patient outcomes?

Although efficacy data associated with CoQ10 treatment are somewhat limited, the remarkable safety profile should encourage further clinical studies. Currently several clinical trials are in progress which will help evaluate CoQ10 efficacy in pediatric and adult patient populations. Awareness

of the importance of uptake and distribution factors which affect CoQ10 may contribute to the success of future trials.

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Review

Plasma coenzyme Q10 response to oral ingestion of coenzyme Q10 formulations

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Abstract

Plasma coenzyme Q10 (CoQ10) response to oral ingestion of various CoQ10 formulations was examined. Both total plasma CoQ10 and net increase over baseline CoQ10 concentrations show a gradual increase with increasing doses of CoQ10. Plasma CoQ10 concentrations plateau at a dose of 2400 mg using one specific chewable tablet formulation. The efficiency of absorption decreases as the dose increases. About 95% of circulating CoQ10 occurs as ubiquinol, with no appreciable change in the ratio following CoQ10 ingestion. Higher plasma CoQ10 concentrations are necessary to facilitate uptake by peripheral tissues and also the brain. Solubilized formulations of CoQ10 (both ubiquinone and ubiquinol) have superior bioavailability as evidenced by their enhanced plasma CoQ10 responses.
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1. Introduction

Coenzyme Q10 (CoQ10), also known as ubiquinone or ubidecarenone, is a naturally occurring compound with a ubiquitous distribution in nature. Structurally it is similar to vitamin K and its chemical nomenclature is 2,3-dimethoxy-5-methyl-6-decaprenyl-1,4-benzoquinone (*trans* configuration). CoQ10 functions like a vitamin in the body, but it is not considered one because unlike vitamins it is synthesized in the body. CoQ10 has a fundamental role in cellular bioenergetics as a cofactor in the mitochondrial electron transport chain and is essential for the production of ATP (Ernster and Dallner, 1995). The functions of CoQ10 in the body go beyond its role in the mitochondria. CoQ10 in its reduced form as the hydroquinone (ubiquinol) is a potent lipophilic antioxidant and thus protects intra- and extra-cellular components from free radical damage. As an antioxidant, CoQ10 is also capable of recycling and regenerating other antioxidants such as tocopherol and ascorbate. In addition, other important functions of CoQ10 such as cell signaling and gene expression have been recognized (Ernster and Dallner, 1995; Crane, 2001; Groneberg et al., 2005).

Numerous health benefits of CoQ10 supplementation have been reported in the literature. A large number of these studies relate to cardiovascular diseases where CoQ10 has been used as an adjunct to standard medical therapy (Greenberg and Frishman, 1990; Overvad et al., 1999; Langsjoen and Langsjoen, 1999; Belardinelli et al., 2006). There is also evidence for its beneficial effect in pediatric cardiomyopathy (Elshershari et al., 2003; Bhagavan and Chopra, 2005). In recent years, CoQ10 is being tested as a therapeutic agent in several neurodegenerative diseases. The importance of CoQ10 in the treatment of mitochondrial diseases is now recognized (DiMauro et al., 2006). Preliminary results with CoQ10 in the treatment of neurologic diseases such as Parkinson's and Huntington's are promising (Kieburtz, 2001; Beal, 2002; Shults et al., 2002; Shults, 2003). The potential therapeutic value of CoQ10 has also become evident in several other conditions (Littarru and Tiano, 2005; Bhagavan and Chopra, 2006).

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Most of the beneficial effects of CoQ10 may be attributed to its fundamental role in mitochondrial energy production.

The purpose of this review is to examine plasma CoQ10 response to orally ingested CoQ10 formulations as an indicator of CoQ10 bioavailability in these products. CoQ10 is available over the counter in various product forms as a dietary supplement in the US and elsewhere, and it has become increasingly popular. CoQ10 is also available as a drug in a few countries. CoQ10 in its pure form is a powder (crystalline) that is insoluble in water and has limited solubility in lipids, and therefore it is poorly absorbed. The importance of product formulation on CoQ10 bioavailability has been suggested previously (Chopra et al., 1998). CoQ10 products currently available on the market include powder-based compressed tablets, chewable tablets, powder-filled hard-shell capsules and softgels containing an oil suspension. The rationale for the latter is that the presence of fat may promote better absorption of CoQ10 since it is lipophilic. In addition, several solubilized formulations of CoQ10 in softgel and liquid forms have become available in recent years. While there is a choice of dosage forms available, one major issue concerning their use whether as a dietary supplement for general well-being or for therapeutic purposes is their potential efficacy. An important determinant of efficacy is absorption/bioavailability of CoQ10 in the various products. Unfortunately, most consumers and also health care professionals are generally unaware of the importance of bioavailability especially as it relates to poorly soluble dietary supplements such as CoQ10. This review deals with data from controlled clinical trials on plasma CoQ10 response to oral ingestion CoQ10 products in order to ascertain which product types have superior bioavailability.

2. Data collection

The data collected in this report are based on controlled clinical trials where plasma CoQ10 data following oral ingestion of various types of CoQ10 formulations are available. The increase in plasma CoQ10 in terms of "fold" increase over baseline and also net increase per 100 mg CoQ10 ingested are taken as indicators of their absorption efficiency. In most of the early clinical trials on CoQ10, plasma CoQ10 determinations were not made. In a few studies where it was done, the analytical procedures employed lacked specificity, sensitivity and precision. Trials carried out beginning in the 1980s have employed HPLC methodology in practically all cases, and studies for this review were selected from this group.

The studies are divided into four distinct categories. The first three deals with chronic low/moderate dose studies, chronic high dose studies, and single dose/pharmacokinetic studies. These studies have all utilized powder-based CoQ10 dosage forms comprised of compressed tablets, chewable tablets, powder-filled capsules, and softgel capsules containing an oil suspension. The fourth category deals with a comparison of the powder-based non-solubilized products with the newer solubilized CoQ10 formulations.

The differentiation of low/moderate and high dose was somewhat arbitrary, with the low/moderate dose range covering a dose of 30 mg to 300 mg a day and the high dose range from 300 mg to 3000 mg a day. One criterion for inclusion in the chronic dosing studies was a minimum of two weeks duration since this assured steady state concentrations of plasma CoQ10 (Tomono et al., 1986; Hosoe et al., 2007). Since there are a large number of studies in the low/moderate range, the selection of the limited number of studies for this evaluation was made randomly. Some of the data shown were extrapolated from figures and these are indicated in the tables. While most of the data in this review relate to studies using CoQ10 as ubiquinone, there are two recent studies that have employed the reduced form of CoQ10 as ubiquinol and these studies are discussed separately.

3. Results

3.1. Studies using CoQ10 as ubiquinone

3.1.1. Chronic low/moderate dose studies

A limited number of studies were randomly selected from a large pool for evaluation of plasma CoQ10 response (Table 1) and they represent typical data in this dose range (30–300 mg for two weeks to nine months). Data for both healthy subjects and patients (mostly CHF) are shown separately in the table. The increase in plasma CoQ10 concentration over baseline in terms of multiples of baseline values (expressed as "fold" increase) was calculated by dividing the final value by the baseline value and this ranged from 1.470 to 4.074. A definite increase with increasing doses of CoQ10 was evident. When the data were calculated in terms of net increase per 100 mg of CoQ10 ingested, there was an overall decreasing trend with increasing dose, with no difference between healthy subjects and patients.

3.1.2. Chronic high dose studies

There were several studies in this group where the dosages of CoQ10 ranged from 300 mg to 3000 mg. Data from three studies using escalating doses of one specific chewable tablet formulations of CoQ10 are shown in Table 2 (Shults et al., 1998, 2002, 2004). As could be expected, the increase in plasma CoQ10 concentration over baseline was much greater with high doses of CoQ10 than that with low/moderate doses of CoQ10. On the other hand, the increase in plasma CoQ10 per 100 mg of CoQ10 ingested was much lower than that with lower doses indicating decreased efficiency of absorption, and this value did not change with increasing dosages in the high dose range.

In one trial using CoQ10 powder as an oil suspension at doses ranging from 300 mg to 900 mg (Ikematsu et al., 2006), the increment in plasma CoQ10 over the baseline

Table 1
Plasma CoQ10 response to chronic ingestion of low/moderate dose CoQ10 in adults using tablets, powder-filled capsules or oil-suspensions

Total daily dose, mg (duration)	N	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg	References
		Baseline	Final			
Normal subjects:						
30 (oil susp.) (2 months)	28	1.355	1.992	1.470	2.123	Zita et al. (2003)
50 (powder) (15 days)	20	1.019	1.587	1.557	1.136	Lu et al. (2003)
90 (oil susp.) (2 weeks)	22	0.700[a]	1.950[a]	2.786	1.389	Weber et al. (1994)
90 (oil susp.) (2 months)	20	1.070	2.970	2.776	2.111	Kaikkonen et al. (1997)
90 (powder) (2 months)	20	1.080	2.890	2.676	2.011	Kaikkonen et al. (1997)
100 (oil susp.)[b] (2 weeks)	5	0.656	1.180	1.799	0.524	Lonnrot et al. (1996)
100 (oil susp.) (2 months)	36	1.355	2.930	2.162	1.575	Zita et al. (2003)
200 (oil susp.) (20 days)	15	0.695	2.085	3.000	0.695	Serebruany et al. (1997)
Patients (mostly CHF):						
99 (tablets) (3 months)	27	0.903	2.029	2.247	1.137	Watson et al. (1999)
100 (oil susp.) (3 months)	69	1.170	2.780	2.376	1.610	Hofman-Bang et al. (1995)
100 (oil susp.) (3 months)	17	1.042	2.317	2.224	1.275	Henriksen et al. (1999)
200 (oil susp.) (6 months)	23	1.100	2.548	2.316	0.724	Khatta et al. (2000)
300 (oil susp.) (2 weeks)	62	0.452	1.842	4.074	0.463	Rosenfeldt et al. (2005)
300 (oil susp.) (4 weeks)	23	0.950	3.764	3.962	0.938	Belardinelli et al. (2006)

[a] Extrapolated from figure.
[b] With 500 mg vitamin C.

Table 2
Plasma CoQ10 response to chronic ingestion of high-dose CoQ10 in patients with Parkinson's disease using specific chewable tablet formulations[a]

Total daily dose (mg)	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	Final		
Shults et al. (1998); *N* = 15; Duration: 1 month (Data extrapolated from figure)				
Placebo	0.680	0.680	1.000	–
400		2.490	3.650	0.450
600		2.900	4.240	0.370
800		3.590	5.260	0.360
Shults et al. (2002); *N* = 80; Duration: 16 months (Data extrapolated from figure)				
Placebo	0.580	0.580	1.000	–
300		1.910	3.310	0.450
600		2.550	4.420	0.330
1200		4.580	7.930	0.330
Shults et al. (2004); *N* = 17; Duration: 2 weeks (Data extrapolated from figure)				
1200	1.160	4.630	4.000	0.290
1800		7.180	6.200	0.340
2400		8.690	7.500	0.310
3000		8.690	7.500	0.250

[a] The chewable CoQ10 tablets used in the above studies contained varying amounts of vitamin E, the total ranging from 800 IU to 1500 IU depending on the dose of CoQ10.

showed a slight increase with increasing doses of CoQ10 whereas the increase per 100 mg CoQ10 ingested showed a definite decline with increasing doses of CoQ10 (Table 3). The data are somewhat similar to the results obtained with high dose chewable tablet formulations (Table 2).

3.1.3. Single dose studies

There were several studies in this category and data from four trials covering a dose range of 30 mg to 333 mg are shown in Table 4. The increase per 100 mg values were calculated using the C_{max} data. As in the case of the chronic studies, the increment (fold) in plasma CoQ10 values showed an increase as the dose increased whereas the increase per 100 mg CoQ10 ingested tended to decrease, similar to the findings in the chronic low/moderate dose studies.

3.1.4. Comparison of solubilized vs. powder-based formulations: chronic dose studies

Data from two studies (Chopra et al., 1998) are shown in Table 5. In the first, a solubilized CoQ10 softgel formulation (Q-Gel®) was compared with tablets, powder-filled hard shell capsules and softgels containing an oil suspension. Both the increase (fold) and increase per 100 mg were markedly higher for the solubilized formulation as compared with powder-based non-solubilized formulations.

Table 3
Plasma CoQ10 response to chronic ingestion of high-dose CoQ10 in healthy adults using a softgel formulation containing CoQ10 as an oil suspension

Total daily dose (mg)	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	Final		
Ikematsu et al. (2006) $N = 88$; Duration: 4 weeks (Data extrapolated from figure)				
300	0.695	3.070	4.420	0.790
600		3.590	5.170	0.480
900		3.710	5.330	0.340

In the second study, only the oil suspension was compared with the solubilized CoQ10 formulation and the results were very similar.

3.1.5. Comparison of solubilized vs. powder-based formulations: single dose studies

A pharmacokinetic evaluation of three solubilized formulations of CoQ10 as compared with a powder-filled hard shell capsule formulation was carried out by Miles et al. (2002). The three solubilized products included a CoQ10 formulation as a syrup (LiQ10®), a softgel formulation (Q-Gel®) and a softgel formulation containing CoQ10 in its reduced form as ubiquinol (Q-Nol®). The three solubilized formulations were found to be far superior to the powder-filled capsules in terms of both increase (fold)

and increase per 100 mg, and solubilized ubiquinol was found to be the best of the three solubilized formulations (Table 6).

Molyneux et al. (2004) conducted a single dose study with a number of marketed CoQ10 products using $C_{\max}$ (6 h) for comparison (Table 7). Of these, six were non-solubilized formulations (oil suspensions, powder-filled capsules and chewable tablets) and one was a solubilized formulation (Q-Gel®). Again in this study, plasma CoQ10 response was found to be much higher for the solubilized formulation as compared with the non-solubilized formulations.

3.2. Studies using CoQ10 as ubiquinol

3.2.1. Chronic low/moderate dose studies using ubiquinol

Data from a recent study are available where the plasma CoQ10 concentrations were determined following supplementation with 90 mg, 150 mg, and 300 mg of CoQ10 as ubiquinol as an oil suspension for four weeks (Hosoe et al., 2007). This was a dose ranging study, and there was no comparison with any other product. There was a gradual and somewhat linear increase in plasma CoQ10 concentrations with increasing dose. At a daily dose of 300 mg ubiquinol for 4 weeks, plasma ubiquinol concentration reached a markedly high value of 8.413 μmol/L, an 11-fold increase over baseline (Table 8). Likewise, the increase per 100 mg values was also remarkably high compared

Table 4
Plasma CoQ10 response to ingestion of a single low/moderate dose CoQ10 in healthy adults using powder-filled capsules/tablets or oil-suspensions

Dose (mg)	N	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg	References
		Baseline	$C_{\max}$[a]			
30 (oil susp.)	9	1.019	0.359	1.352	1.197	Weber et al. (1997a)
100 (powder)	16		1.163		1.163	Tomono et al. (1986)
100 (oil susp.1)	10		1.330[b]		1.330	Weis et al. (1994)
100 (oil susp.2)	10		0.700[b]		0.700	Weis et al. (1994)
100 (oil susp.3)	10		0.760[b]		0.760	Weis et al. (1994)
100 (powder)	10		0.750[b]		0.750	Weis et al. (1994)
333 (powder)	8	1.645	2.783	2.692	0.808	Lucker et al. (1984)

[a] Corrected for baseline.
[b] Extrapolated from figure.

Table 5
Plasma CoQ10 response to chronic ingestion of low/moderate dose CoQ10 in adults using solubilized and powder-based formulations

Formulation	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	Final		
Chopra et al. (1998); Study 1: $N = 24$; Dose: 120 mg; Duration: 3 weeks				
Solubilized (softgel caps)[a]	0.579	3.824	6.622	2.713
Oil-suspension	0.579	1.587	2.740	0.840
Tablets	0.602	1.853	3.078	1.043
Powder-filled caps	0.579	1.888	3.261	1.091
Chopra et al. (1998): Study 2: $N = 24$; Dose:120 mg; Duration: 4 weeks				
Solubilized (softgel caps)[a]	0.440	3.243	7.370	2.336
Oil-suspension	0.463	1.459	3.151	0.830

[a] Q-Gel®.

Table 6
Plasma CoQ10 response to ingestion of a single low/moderate dose CoQ10 in adults using solubilized and powder-based formulations

Formulation	Plasma CoQ10 (µmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	C_{max}		
Miles et al. (2002); $N = 9$ (crossover); Dose: 180 mg (2.1 mg/kg)				
Solubilized (syrup)[a]	0.602	1.795	2.982	0.663
Solubilized (softgel caps)[b]	0.602	1.795	2.982	0.663
Solubilized (ubiquinol caps)[c]	0.602	2.073	3.444	0.817
Powder (caps)	0.602	0.741	1.231	0.077

[a] LiQ10®.
[b] Q-Gel®.
[c] Q-Nol®.

Table 7
Plasma CoQ10 response to ingestion of a single low/moderate dose CoQ10 in adults using solubilized and powder-based formulations

Formulation	Plasma CoQ10 (µmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	C_{max}		
Molyneux et al. (2004); $N = 10$ (cross-over); Dose: 150 mg				
Solubilized[a]	0.850	1.436	1.689	0.391
Suspension 1 (softgel caps)	0.850	1.171	1.378	0.214
Suspension 2 (softgel caps)	0.850	1.079	1.269	0.153
Suspension 3 (softgel caps)	0.850	1.027	1.208	0.118
Suspension 4 (softgel caps)	0.850	1.023	1.204	0.115
Powder-filled caps	0.850	1.053	1.239	0.135
Chewable tablets	0.850	0.989	1.164	0.093

[a] Q-Gel®.

with results obtained with both high and low/moderate dose powder-based CoQ10 formulations in the form of ubiquinone.

3.2.2. Chronic low and high dose studies using ubiquinol

In a recent trial, a unique solubilized formulation of CoQ10 in its reduced form as ubiquinol (Li-Q-Nol®) was tested in children at both low and high doses, viz. 1 mg/kg/d and 10 mg/kg/d each for one month duration (Miles et al., 2006). Plasma CoQ10 values, extrapolated to a dosage of 600 mg a day for an adult, 60 kg, for purposes of comparison with adult dosages, are shown in Table 9. The plasma response at this dosage was comparable to data from studies involving much higher doses of CoQ10 as ubiquinone (2400 mg–3000 mg a day). The increase in per 100 mg CoQ10 ingested was also much higher at the 600 mg dose with solubilized ubiquinol as compared with other high dose studies using much higher doses of

powder-based CoQ10 formulations as ubiquinone (Tables 2 and 3).

3.2.3. Single dose studies using ubiquinol

In one study referred to earlier (Miles et al., 2002), a solubilized formulation of ubiquinol was compared with a powder-filled capsule and two solubilized formulations of CoQ10 based on ubiquinone with respect to their pharmacokinetic parameters (Table 6). While the three solubilized formulations were far superior to the powder-based product, solubilized ubiquinol was found to be even better than the two solubilized ubiquinone-based products.

In a very recent study, ubiquinol as an oil suspension was tested at two doses, viz. 150 mg and 300 mg (Hosoe et al., 2007). The C_{max} values (6 h) were 2.173 µmol/L and 3.686 µmol/L, and the increases over plasma baseline concentrations were 2.3-fold and 4.7-fold, respectively (Table 10). Both the net increase in plasma ubiquinol concentration and the increase per 100 mg were higher as com-

Table 8
Plasma CoQ10 response to chronic ingestion of low/moderate dose CoQ10 as ubiquinol[a] in adults

Total daily dose (mg)	Plasma CoQ10 (µmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	Final		
Hosoe et al. (2007); Duration: 4 weeks				
90 (20)[b]	0.659	3.282	4.981	2.926
150 (20)	0.751	4.437	5.908	2.457
300 (19)	0.763	8.413	11.026	2.550

[a] Softgel formulation containing emulsified CoQ10 as ubiquinol (Kaneka QH™).
[b] Figures in parentheses indicate the number of subjects.

Table 9
Plasma CoQ10 response to chronic ingestion of high-dose CoQ10 in children using a liquid formulation containing solubilized CoQ10 as ubiquinol (Li-Q-Nol®)[a]

Total daily dose (mg/kg/d)	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg[b]
	Baseline	Final		
Miles et al. (2006); $N = 16$; Duration: 1 month (Data extrapolated from figure)				
1 (run-in)	0.950	3.000	3.158	3.416
10 (od)		8.500	8.947	1.258
10 (bid)		10.700	11.263	1.625

[a] A solubilized ubiquinol formulation as a syrup.
[b] Extrapolated to an adult weighing 60 kg; Equivalent adult dose of 60 mg and 600 mg, respectively.

Table 10
Plasma CoQ10 response to ingestion of a single low/moderate dose CoQ10 as ubiquinol[a] in adults

Formulation (mg)	Plasma CoQ10 (μmol/L)		Increase (fold)	Increase per 100 mg
	Baseline	C_{max}		
Hosoe et al. (2007); $N = 15$				
150	0.945	2.173	2.300	0.819
300	0.784	3.686	4.702	0.964

[a] Softgel formulation containing emulsified CoQ10 (Kaneka QH™).

pared with single dose studies using non-solubilized CoQ10 formulations in the form of ubiquinone at similar dosages.

4. Discussion

4.1. Studies using CoQ10 as ubiquinone

4.1.1. Chronic low/moderate dose studies

The data on plasma responses to low/moderate doses were derived mostly from studies employing CoQ10 as an oil suspension in softgel capsules since these are more commonly available products (Table 1). Overall, there was a gradual increase in plasma CoQ10 values expressed in terms of "fold' increase over baseline CoQ10 concentrations whereas the increase per 100 mg CoQ10 ingested showed a decreasing trend with increasing dosage. This shows that in the low/moderate dose group ranging from 30 mg to 300 mg, the efficiency absorption decreases as the dose increases.

4.1.2. Chronic high dose studies

The data on high dose studies reported in Table 2 were based entirely on the use of one specific powder-based chewable tablet formulation of CoQ10. As could be expected, plasma CoQ10 concentrations and the increment over baseline values showed a gradual increase with increasing dosage of CoQ10 (Tables 2 and 3; data extrapolated from figures), and this response was much greater than that seen with low/moderate doses (Table 1). An examination of the data covering the entire dose range,

from 30 mg to 3000 mg, shows that the plasma concentrations tend to plateau at around 2400 mg with no further increase at 3000 mg, using one specific chewable tablet formulation of CoQ10 (Shults et al., 2004). This was confirmed in a subsequent study using the same chewable tablet formulation of CoQ10 (Ferrante et al., 2005).

There was a striking reduction in the net increase in plasma CoQ10 concentration per 100 mg CoQ10 ingested in the high dose studies as compared with the data from low/moderate dose studies. This shows that the efficiency of absorption decreases sharply in the high dose range, and this would be as expected for a fat soluble nutrient such as CoQ10 ingested at pharmacologic doses.

In all the three high dose studies shown in Table 2, the specific CoQ10 chewable tablet formulations used contained rather large amounts of vitamin E contributing to total daily intake ranging from 800 IU to 1500 IU depending upon the dose of CoQ10. Since it is known that high dose vitamin E ingested along with CoQ10 may interfere with CoQ10 absorption and thus result in lower plasma CoQ10 concentrations (Chopra and Bhagavan, 1999; Kaikkonen et al., 2000), the figures on the increase in plasma CoQ10 concentration and in net increase per 100 mg shown in the table may be somewhat underestimated for similar CoQ10 dosage forms of the same strength that do not contain vitamin E.

4.1.3. Single dose studies

The four studies shown in Table 4 are single dose pharmacokinetic studies using low to moderate doses of CoQ10. The overall response pattern to CoQ10 ingestion is quite similar to that seen in the chronic dose studies, i.e., with an increasing dose of CoQ10, an increase in C_{max} and a decrease in net increase per 100 mg. The T_{max} in these studies is consistently around 6 h which shows that CoQ10, a rather large molecule which is water-insoluble, is absorbed slowly in the intestine.

4.1.4. Comparison of solubilized vs. powder-based CoQ10 formulations: chronic dose studies

Bioavailability assessments of a solubilized CoQ10 formulation (Q-Gel®) introduced in the mid 1990s were carried out by Chopra et al. (1998). There were two studies and in the first, it was compared with three non-solubilized formulations (tablets, powder-filled capsules and an oil suspension), and with an oil suspension only in the second study. The results from both the studies clearly showed the enhanced bioavailability of the solubilized CoQ10 formulation.

4.1.5. Comparison of solubilized vs. powder-based CoQ10 formulations: single dose studies

In one study by Miles et al. (2002), the pharmacokinetic profiles of three solubilized CoQ10 formulations were compared with that of a powder-based formulation following a single dose. The solubilized products also included a novel reduced form of CoQ10 as ubiquinol in a softgel

formulation (Q-Nol®). The three solubilized formulations were found to be far superior to the powder-filled capsules in terms of both increase (fold) and net increase per 100 mg ingested, and the solubilized ubiquinol was found to be the best of the three solubilized CoQ10 formulations.

Molyneux et al. (2004) conducted a single dose study with a number of marketed CoQ10 products using a rather simple design. They employed C_{max} (6 h) for comparison of plasma CoQ10 responses as an indicator of bioavailability of the various products. Of the seven products tested, six were powder-based formulations (oil suspensions, powder-filled capsules and chewable tablets) and one was a solubilized formulation (Q-Gel®). The increase in plasma CoQ10 was much higher with the solubilized formulation than that with the powder-based formulations, and this is consistent with previous findings on the superiority of solubilized CoQ10 formulations (Chopra et al., 1998; Miles et al., 2002; Zaghloul et al., 2002).

4.2. Studies using CoQ10 as ubiquinol

4.2.1. Chronic low/moderate dose studies

In a dose-ranging study, Hosoe et al. (2007) administered daily doses of 90 mg, 150 mg, and 300 mg ubiquinol (as an oil suspension in softgel capsules, Kaneka QH™) to healthy adults for four weeks and found remarkably high plasma CoQ10 concentrations (as ubiquinol), up to 11-fold increase over baseline at the 300 mg dose. In their study, plasma ubiquinol accounted for 96%–98.5% of plasma CoQ10 at all the four data points. In terms of increase over baseline (fold) and net increase per 100 mg CoQ10 ingested, these numbers are impressive indicating superior absorption of CoQ10 in the form of ubiquinol (Table 8). The fact that there was only a slight difference between the 90 mg, 150 mg, and 300 mg doses with respect to increase per 100 mg (2.926, 2.457, and 2.550, respectively) indicates that the efficiency of absorption was not appreciably affected with increasing doses of ubiquinol in this dose range. It would be of interest to examine at what point does this begin to show a decrease as the dose increases beyond 300 mg. It may be noted that the data for the 150 mg dose are comparable to those of Chopra et al. (1998) for solubilized CoQ10 formulation as ubiquinone at a dose of 120 mg (Table 5).

4.2.2. Chronic high dose studies

Miles et al. (2006) employed a novel liquid formulation of CoQ10 as ubiquinol in the form of syrup (Li-Q-Nol®) in a dose-ranging study with children (Table 9). While the results were somewhat similar to those with adults in terms of plasma CoQ10 concentrations (Table 2), the plasma CoQ10 response was far greater when compared on the basis of dosage. The comparison was based on an equivalent adult dosage of 600 mg for an individual weighing 60 kg. Furthermore, when the dosage was administered in two divided doses, the response was even higher. This is not surprising and it shows that split-dosing is more

effective especially when administering pharmacologic doses of CoQ10. A similar effect has been observed with CoQ10 administered as ubiquinone (Singh et al., 2005).

The highest net increase in plasma CoQ10 concentration and also the highest increase per 100 mg CoQ10 ingested was observed using solubilized CoQ10 as ubiquinol at a dose of 600 mg (Miles et al., 2006), and these values are higher than those obtained with much larger doses of CoQ10 (up to 3000 mg) as ubiquinone. According to the authors, after correcting for body weight differences, the dosage of CoQ10 (as ubiquinol) employed in their study was approximately 3-fold lower than that reported by Shults et al. (2004) using a specific chewable CoQ10 tablet formulation at a daily dose of 2400 mg.

Incidentally, the highest plasma CoQ10 concentration reported in the literature thus far is 10.7 µmol/L that was achieved using CoQ10 in the form of ubiquinol (Miles et al., 2006). Whether this represents a value close to a ceiling for plasma CoQ10 needs to be established. Furthermore, it would be important to determine whether such high plasma concentrations afford maximum therapeutic benefit.

4.2.3. Single dose studies

In the pharmacokinetic study by Miles et al. (2002), solubilized ubiquinol in a softgel formulation was one of the products tested along with two solubilized CoQ10 formulations as ubiquinone and a powder-filled capsule (Table 6). While the solubilized CoQ10 formulations were found to be far superior to the powder-based product, solubilized ubiquinol was found to be the best of the three solubilized formulations in terms of pharmacokinetic profile, increase over baseline (fold) and net increase per 100 mg CoQ10 ingested.

The single dose study by Hosoe et al. (2007) employed ubiquinol as an oil suspension (Kaneka QH™) at two doses, viz. 150 mg and 300 mg (Table 10). The plasma CoQ10 (ubiquinol) response at C_{max} (6 h) was higher than that in studies using CoQ10 as ubiquinone (Tables 4 and 6). The data for the 150 mg dose is comparable to the ubiquinol data of Miles et al. (2002) at a dose of 180 mg (Table 6).

Both the chronic dose and single dose studies clearly demonstrate that solubilized formulations of CoQ10 whether in the form of ubiquinone or as ubiquinol are superior to non-solubilized CoQ10 formulations in terms of plasma CoQ10 response. This reflects the superior bioavailability of solubilized CoQ10 formulations, and solubilized ubiquinol appears to be even better than solubilized ubiquinone. With non-solubilized formulations of CoQ10, data show that ubiquinol is superior to ubiquinone.

4.3. Pharmacokinetic profile of CoQ10

4.3.1. Ubiquinone

There are several studies that provide data on the pharmacokinetic parameters of orally ingested CoQ10 in the

form of ubiquinone. The paper by Tomono et al. (1986) that is cited often has provided useful basic information on the pharmacokinetic parameters of ubiquinone. A T_{max} of about 6 h for ubiquinone has been confirmed by others (Weis et al., 1994; Weber et al., 1997a; Miles et al., 2002). This shows that CoQ10 is slowly absorbed in the GI tract and this is attributable to its hydrophobicity and relatively large molecular weight. The T_{max} is apparently not affected in the case of sustained release tablets (Lu et al., 2003). The steady-state T_{max} appears to be somewhat lower at about 5 h (Lucker et al., 1984). A second plasma CoQ10 peak has been observed at about 24 h following oral ingestion of ubiquinone (Lucker et al., 1984; Tomono et al., 1986; Weis et al., 1994) which could be attributed to both enterohepatic recycling and redistribution from the liver to circulation primarily via the LDL/VLDL fractions.

4.3.2. Ubiquinol

Solubilized and stabilized formulations of ubiquinol became available a few years ago. Miles et al. (2002) conducted the first pharmacokinetic study with ubiquinol using a softgel formulation along with three other products based on ubiquinone. The pharmacokinetic profile of ubiquinol was identical to that of ubiquinone with a T_{max} at 6 h except that the plasma response of ubiquinol was much greater. There was also a shoulder at 24 h that is indicative of a second peak. Hosoe et al. (2007) have confirmed these findings on ubiquinol and furthermore, their data is suggestive of an additional shoulder peak at 12 h. Although the elimination half-life of ubiquinol could not be accurately determined in their study, it was estimated to be about 48 h.

That the pharmacokinetic profiles of ubiquinone and ubiquinol are identical is not surprising due to the fact that circulating CoQ10 is almost entirely in the form of ubiquinol (Yamashita and Yamamoto, 1997; Tang et al., 2001; Miles et al., 2003) and that the conversion of ubiquinone to ubiquinol occurs in the enterocytes prior to its lymphatic transport into circulation (Mohr et al., 1999; Craft et al., 2005; Bhagavan et al., 2007).

4.4. Other factors influencing plasma CoQ10 concentrations

Apart from the expected increase in plasma CoQ10 concentrations following oral ingestion of CoQ10 formulations, there are other factors that have an influence on plasma CoQ10 values. Dietary fat is known to improve CoQ10 absorption. Dietary contribution of CoQ10 is minimal unless one is consuming rather large quantities of organ meats such as cooked pork heart (Weber et al., 1997a). The intake of CoQ10 from a typical Western diet has been estimated to be about 3–5 mg a day, primarily derived from meat and poultry (Weber et al., 1997b). There is evidence to show that high dose vitamin E ingested along with CoQ10 may interfere with CoQ10 absorption and thus result in lower plasma CoQ10 (Chopra and Bhagavan, 1999; Kaikkonen et al., 2000), This may be due to compe-

tition during the absorption process in the intestine. Other determinants of plasma CoQ10 include serum cholesterol, serum triglycerides, gender, alcohol consumption, and age (Kaikkonen et al., 1999, 2002).

There is evidence to show that exogenously administered CoQ10 does not down-regulate endogenous synthesis of CoQ10. This is borne out by the fact that plasma CoQ10 concentrations return to their baseline values but not lower after cessation of CoQ10 supplementation regardless of the dose whether as ubiquinone (Tomono et al., 1986; Ikematsu et al., 2006) or as ubiquinol (Hosoe et al., 2007). This finding is consistent with previous data from animal studies (Zhang et al., 1995).

4.5. Plasma CoQ10 measurements in clinical trials

The importance of monitoring plasma CoQ10 concentrations in clinical trials involving pharmacologic doses of CoQ10 cannot be overemphasized (Bhagavan et al., 2001; Steele et al., 2004). Higher than "normal" plasma CoQ10 concentrations appear to be necessary to promote uptake by peripheral tissues and also to cross the blood brain barrier. The plasma threshold for the uptake of CoQ10 appears to be different for different tissues. For instance, in one study with congestive heart failure patients, it was reported that those with a plasma CoQ10 value of 2.4 μg/mL (2.780 μmol/L) showed the highest benefit (Belardinelli et al., 2006). In an earlier study with CHF patients, it was reported that a blood CoQ10 concentration of at least 3.5 μg/mL (4.054 μmol/L) appeared to be necessary before any therapeutic benefit from CoQ10 supplementation could be expected (Langsjoen and Langsjoen, 1998). The plasma threshold appears to be much higher for neurodegenerative diseases such as Huntington's (Kieburtz, 2001) and Parkinson's (Shults et al., 2002), based upon the CoQ10 dosages required to achieve clinical response and also on blood CoQ10 data where available (Shults et al., 2002).

In this context, it may be noted that there are a few studies often cited in the literature where the beneficial effects of CoQ10 supplementation in heart disease could not be demonstrated (Watson et al., 1999; Khatta et al., 2000; Permanetter et al., 1992). Plasma CoQ10 data following CoQ10 supplementation are available only for the first two studies (2.029 μmol/L for Watson et al. and 2.548 μmol/L for Khatta et al.) and both are below the indicated threshold for heart disease patients. It therefore appears that this could have been at least one factor contributing to the lack of beneficial effect of CoQ10 in these studies, which could be attributed to both the dosage and also the bioavailability of the products used.

4.6. Redox status of plasma CoQ10

Plasma CoQ10 is present almost entirely (about 95%) in its reduced form as ubiquinol in healthy subjects (Yamashita and Yamamoto, 1997; Tang et al., 2001; Miles

et al., 2003). The redox status is not affected by gender or race (Miles et al., 2003). Furthermore, orally ingested CoQ10, whether as ubiquinone or as ubiquinol and regardless of the dose, appears in circulation as ubiquinol with no change or very little change in its redox status (Kaikkonen et al., 2000; Mohr et al., 1992; Weber et al., 1994; Hosoe et al., 2007). This shows that there is an efficient mechanism to convert orally administered CoQ10 as ubiquinone to ubiquinol in vivo. There is evidence to show that this reduction takes place in the intestine following absorption before CoQ10 enters the lymphatic system. This was demonstrated in a recent study using Caco-2 cells in culture that the reduction occurs in the enterocytes (Craft et al., 2005; Bhagavan et al., 2007). It was shown in an earlier experiment with rats that orally administered CoQ9 (major homolog in rats) and also CoQ10 were recovered as the corresponding ubiquinols in mesenteric lymph, thus demonstrating their reduction to corresponding ubiquinols in the intestine (Mohr et al., 1999). It is of interest to note in this context that the redox status of CoQ10 in plasma may serve as a sensitive biomarker for oxidative stress (Yamashita and Yamamoto, 1997; Miles et al., 2005).

5. Summary

Plasma CoQ10 concentrations and also the net increase over baseline plasma CoQ10 values show a gradual increase with increasing dose of CoQ10 from low/moderate to high doses. Not surprisingly, the efficiency of absorption decreases as the dose increases, and this is particularly striking at high doses. Split dosing is superior to single dosing with pharmacologic doses of CoQ10. Plasma CoQ10 concentrations appear to plateau at 2400 mg using one specific chewable tablet formulation of CoQ10.

Highest plasma CoQ10 concentration reported thus far is 10.7 µmol/L using a solubilized ubiquinol formulation. Whether this value is close to a ceiling for plasma CoQ10 is not known at this time. Furthermore, whether such high plasma concentrations maximize the therapeutic potential of CoQ10 needs to be explored.

About 95% of circulating CoQ10 is present in its reduced form as ubiquinol in healthy subjects and this ratio is not affected by oral ingestion of CoQ10 either as ubiquinone or as ubiquinol. Plasma redox status of CoQ10 appears to be a sensitive biomarker for oxidative stress.

Plasma CoQ10 concentrations need to be high (i.e. higher than ''normal'' values) in order to promote uptake by peripheral tissues and possibly also to cross the blood brain barrier. The plasma threshold for uptake appears to be different for different tissues. Among non-solubilized formulations of CoQ10, ubiquinol has been found to be superior to ubiquinone in its plasma CoQ10 response. The response following ingestion of solubilized formulations of CoQ10 is much greater indicating their superior bioavailability as compared with non-solubilized powder-based CoQ10 products (compressed tablets, chewable tablets, powder-filled capsules, and softgels containing a suspension in oil). Solubilized formulations of ubiquinol appear to be even better than solubilized ubiquinone. Thus, comparably high or even higher plasma CoQ10 concentrations may be achieved using much lower doses of the solubilized CoQ10 formulations, and this is of particular importance in neurodegenerative diseases where higher plasma concentrations appear to be necessary for therapeutic benefit. The beneficial effects of CoQ10 may be largely attributed to its fundamental role in mitochondrial function and cellular bioenergetics.

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Analysis of coenzyme Q in human blood and tissues

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Abstract

The major coenzyme Q species in humans is the decaprenyl quinoid derivative coenzyme Q_{10} (CoQ_{10}), and its measurement is somewhat challenging owing to its hydrophobicity and tendency to be oxidized. There are three major methods which are suited for analysis of CoQ_{10}: HPLC-coupled UV or electrochemical detection, and tandem mass spectrometry. The techniques are discussed, and results of these applications to determine CoQ_{10} concentrations in various human fluids and tissues are summarized.
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Keywords: Coenzyme Q_{10}; Electrochemical detection; Tandem mass spectrometry; Chromatography

1. Introduction

Coenzyme Q_{10} (ubiquinone, CoQ_{10}) is the principal physiologic quinoid in humans, composed of a 1,4-benzoquinone ring substituted with 3-methyl and 5,6-methoxy groups, and a decaprenyl side chain at position 6 (IUPAC-IUB Commission, 1975). It is integrated in the mitochondrial oxidative phosphorylation system, acting as an electron carrier from complex I (NADH ubiquinone reductase), complex II (succinate ubiquinone reductase) and electron transfer flavoprotein oxidoreductase (from the flavin-dependent dehydrogenases of fatty acid β-oxidation and other metabolic pathways) to complex III (ubiquinol:cytochrome *c* reductase) in the mitochondrial respiratory chain. It is also a transmembrane hydrogen carrier from the mitochondrial matrix to the intermembrane space. Two intrinsic molecular properties necessary for its function – the hydrophobicity of its polyprenyl side chain and the facile oxidation of its benzoquinone ring – make the analysis of CoQ_{10} technically challenging. The measurement of CoQ_{10} in biological fluids and tissues is of clinical importance in detecting CoQ_{10} deficiency states and for monitoring the uptake and distribution of CoQ_{10} administered as a therapeutic supplement.

2. Analytical methods

2.1. Extraction

Classical methods involved extraction in hexane (Okamoto et al., 1988; Grossi et al., 1992; Kommuru et al., 1998; Wakabayashi et al., 1994; Finckh et al., 1995), or hexane with methanol (Takada et al., 1982; Yamashita and Yamamoto, 1997) or with ethanol (Wang et al., 1999). Solid-phase extraction has also been used for solvent exchange (Grossi et al., 1992), and systems for on-line solid-phase extraction have been developed (Battino et al., 2004). Most newer methods have used 1-propanol, which allows a single step of extraction and direct injection (Tang et al., 2001; Mosca et al., 2002; Littarru et al., 2004; Jiang et al., 2004). 1-Propanol has the advantage of being the most lipophilic alcohol which is miscible with water, and it is a slightly more efficient extraction medium than 2-propanol, and far more effective than lower alcohols (Edlund, 1988).

2.2. Internal standards

The commercial unavailability of non-physiologic internal standards is a major issue, and naturally occurring ubiquinones have been used as internal standards in the assay of CoQ_{10}. The most commonly used internal

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standard is CoQ_9, but other species have also been used, including CoQ_7 (Finckh et al., 1995; Colome et al., 2002). Human plasma does contain small amounts of CoQ_9 which are generally around 1% of CoQ_{10} but may be more significant in occasional samples (Tang et al., 2001), and human muscle contains significant amounts of CoQ_9, so there are limitations to its use as an internal standard. For analysis of muscle, CoQ_6 is a more appropriate internal standard (Tang et al., 2004). In general, synthetic non-physiological analogues are preferable, such as diethoxy-CoQ_{10} (Edlund, 1988) and di-propoxy-CoQ_{10} (Duncan et al., 2005). For mass spectrometric methods, stable isotope dilution is strongly recommended; deuterated CoQ_9 has been synthesized and used in this manner (Schaefer et al., 2004).

2.3. Detection

The earlier methods used UV detection, monitoring absorption in the region of 275 nm (Kommuru et al., 1998; Jiang et al., 2004; Boitier et al., 1998). The sensitivity and specificity was greatly improved with the advent of electrochemical detection (ECD) (Ikenoya et al., 1979; Finckh et al., 1995; Grossi et al., 1992). Electrochemical detection using either amperometric (Takada et al., 1982; Wakabayashi et al., 1994; Yamashita and Yamamoto, 1997; Wang et al., 1999) or coulometric (Lang and Packer, 1987; Okamoto et al., 1988; Finckh et al., 1995; Tang et al., 2001) detectors. Using an in-line electrochemical cell enables the detection of ubiquinol and ubiquinone simultaneously and consequently, total CoQ_{10} (Tang et al., 2001; Mosca et al., 2002).

Liquid chromatography-triple quadrupole (tandem) mass spectrometry (LC–MS/MS) was first used in the isolation and elucidation of the structures of nematode quinone biosynthetic intermediates, like rhodoquinone in *Caernohabditis elegans* by positive electrospray ionization tandem mass spectrometry (ESI(+)-MS/MS) (Takamiya et al., 1999), demethoxy-CoQ_9 in the *C. elegans clk-1* mutants or demethoxy-CoQ_6 in yeast, by positive atmospheric pressure chemical ionization tandem mass spectrometry (APCI(+)-MS/MS) (Miyadera et al., 2001; Jonassen et al., 2002; Padilla et al., 2004). APCI has been applied to analyze quinoids of bacterial origin in the positive (Geyer et al., 2004) and negative (APCI(−)-MS/MS) ionization mode (Gao et al., 2003, 2004). A method to measure CoQ_{10} in rat muscle by HPLC-ESI(+)-MS/MS was reported (Schaefer et al., 2004). APCI is generally preferable to ESI because it has greater tolerance to the presence of salts, and so the overall ionization efficiency of CoQ_{10} is superior in physiologic extracts. An interesting approach to overcome that limitation, using methylamine or other 1-alkylamines as additives to the mobile phase, in order to enhance ESI (Teshima and Kondo, 2005) was reported.

Recently, a selective method for analysis of CoQ_{10} in human serum was reported taking advantage of the efficient electron capture by the ubiquinones using negative APCI (Hansen et al., 2004). The method we have used in our laboratory for more than 2 years (Gangoiti and Barshop, 2004) uses APCI(+)-MS/MS.

The use of appropriate scanning functions in the tandem mass spectrometer allow the isomers to be transmitted through the first quadrupole, to be dissociated in the collision cell and finally identified in the third quadrupole by a specific product ion fragment. All the quinones share a benzoquinone ring and an isoprenoid tail in their structure, and fragmentation produces a characteristic product ion (representing the benzylium ion) resulting from the loss of the isoprenoid moiety. In this manner, we have applied precursor ion scanning to detect the different isoprenyl homologues having the same benzoquinone ring (scanning for precursors of the common benzoquinone fragment for each class, i.e., m/z 197.1 for the ubiquinones, m/z 182 for the rhodoquinones, and m/z 167.2 for the demethoxyquinones). Neutral loss scanning can be used to detect the various substituted benzoquinone intermediates, having the same number of isoprenyl units, since the final reactions in the CoQ_{10} biosynthetic pathway involve changes in the aromatic ring functionalities. Negative mode APCI is more selective than positive APCI, but the transition monitored (loss of a methyl group) does not allow the different scanning experiments between the detectors.

2.4. Chromatography

Because of the hydrophobicity of ubiquinones, reverse-phase chromatography is very successful, and essentially all of the published methods of analysis have used reverse-phase high performance liquid chromatography (RP-HPLC) with C18 stationary phases (or C8 (Sohmiya et al., 2004)). In almost all cases the elution has been isocratic, and the mobile phases have been composed primarily of lower alcohols. Methods using electrochemical detection have included perchlorate (Wang et al., 1999; Mosca et al., 2002) or acetate (Grossi et al., 1992; Tang et al., 2001) as supporting electrolytes. Methods using MS have used ammonium acetate to aid deprotonation in negative mode ionization (Hansen et al., 2004), or formic acid to aid protonation in positive mode ionization (Gangoiti and Barshop, 2004).

A unique approach which could in principle provide an alternative to chromatography has been described (Hagerman et al., 2003), using a synthetic 14-mer peptide containing 10 residues from the sequence of the ubiquinone-binding domain of bovine cytochrome *c* reductase. However, that has only been applied to a system with spectrophotometric detection, and details involving the immobilization of the binding peptide have not been fully developed.

3. Measurement of CoQ_{10} in fluids and tissues

3.1. Plasma

Concentrations of total CoQ_{10} in plasma of healthy subjects have been reported in the range (means ± SD) of

0.675 ± 0.315 (Duncan et al., 2005), 0.75 ± 0.22 (Sohmiya et al., 2004), and $1.11 \pm 0.24 \, \mu mol/L$ (increasing to 4.00 ± 1.88 after chronic supplementation with 3 mg/kg) (Niklowitz et al., 2002) Since CoQ_{10} is known to bind to lipoproteins, the amount of CoQ_{10} in plasma is related to the amount of cholesterol, and differences in total CoQ_{10} may be normalized to total cholesterol (Menke et al., 2004). Concentrations of $0.84 \pm 0.29 \, \mu mol/L$ ($0.255 \pm 0.091 \, \mu mol/mmol$ cholesterol) were reported in healthy children (Niklowitz et al., 2004). Miles et al. (Miles et al., 2004) characterized plasma CoQ_{10} concentrations across ages, finding that the total CoQ_{10} concentration in plasma samples ($1.06 \pm 0.32 \, \mu mol/L$) from young children (0.2–7.6 years) was similar to that ($0.88 \pm 0.26 \, \mu mol/L$) in older children (10.4–17.4 years) and ($1.04 \pm 0.33 \, \mu mol/L$) in adults (29–79 years old), but the concentration adjusted for cholesterol decreased in a statistically significant manner with increasing age ($0.29 \pm 0.10 \, mmol/mol$ cholesterol in younger children, $0.20 \pm 0.05 \, mmol/mol$ in older children, and $0.20 \pm 0.05 \, mmol/mol$ in adults), and similar changes were observed when the plasma CoQ_{10} was normalized for low density lipoprotein (LDL) or for the sum of total cholesterol and triglycerides. Diurnal variation of plasma CoQ_{10} has been studied and reported to be insignificant, although there is a nocturnal decrease, which is correlated with cholesterol concentration (Niklowitz et al., 2006).

3.2. Blood cells

Concentrations of CoQ_{10} in erythrocytes average about 3% of plasma levels. Concentrations of $22.20 \pm 6.17 \, pmol/10^9$ erythrocytes were reported (Niklowitz et al., 2002), with no increase after supplementation of normal subjects, but considerably higher values (280–1093 $pmol/10^9$ cells) in patients with sickle cell anemia (Niklowitz et al., 2002). Platelet total CoQ_{10} content has been reported as $198 \pm 76 \, pmol/10^9$ cells, and (in contrast with the situation in erythrocytes) the content of platelet CoQ_{10} was seen to increase with supplementation (Niklowitz et al., 2004). Mononuclear cells were reported to have CoQ_{10} concentrations of $65 \pm 24 \, pmol/mg$ protein (Duncan et al., 2005).

3.3. Muscle

Skeletal muscle CoQ_{10} content was reported as $33.3 \pm 5.3 \, nmol/g$ wet weight ($28.7 \pm 4.6 \, \mu g/g$) (Lamperti et al., 2003) and $22.0 \pm 5.6 \, nmol/g$ ($38.6 \pm 5.6 \, \mu g/g$) (Boitier et al., 1998) in biopsies from healthy subjects, using UV detection. More recently, total CoQ_{10} content of biopsies from reportedly normal pediatric patients was found to be $18.34 \pm 5.48 \, nmol/g$ ($15.8 \pm 4.7 \, \mu g/g$) tissue (Pastore et al., 2005) and $32.1 \pm 6.8 \, \mu g/g$ (Lopez et al., 2006), using ECD. A subset of patients with cerebellar ataxia were found to have markedly reduced muscle CoQ_{10} concentrations (Lamperti et al., 2003; Musumeci et al., 2001), and functional deficiency of CoQ_{10} is manifest in such patients as low activities of complex I + III and II + III (Boitier

et al., 1998), which in some cases has been shown to be due to specific defects in CoQ_{10} synthesis (Lopez et al., 2006; Quinzii et al., 2006).

3.4. Other tissues

CoQ_{10} was measured in human seminal plasma was determined by UV detection, and reported to be in the range of 37.2–48.5 ng/mL (0.043–0.056 $\mu mol/L$). Concentrations of CoQ_{10} in human breast milk were determined to be $0.32 \pm 9.21 \, \mu mol/L$ (Tang et al., 2006).

4. Determination of reduced and oxidized fractions

CoQ_{10} is known to be carried in circulation by lipoproteins, and is the first antioxidant to be depleted when LDL is subjected to oxidative stress in vitro (Stocker et al., 1991). Altered ratios of ubiquinol ($CoQ_{10}H_2$) to ubiquinone (CoQ_{10}), reflecting greater oxidation (increased CoQ_{10}) have been reported to be present in many disease states, including neonatal hypoxia (Hara et al., 1999), hyperlipidemia and liver disease (Kontush et al., 1997), and Parkinson disease (Buhmann et al., 2004; Sohmiya et al., 2004).

Ubihydroquinone ($CoQ_{10}H_2$) is readily oxidized to ubiquinone (CoQ_{10}), with storage at room temperature or $-20 \, ^\circ C$ (Edlund, 1988) and also in the course of sample preparation, especially when there are extensive steps of extraction (Grossi et al., 1992). Oxidation may proceed as quickly as 4% in plasma during storage for 4 h at 5 °C (Menke et al., 2000), but the redox state generally is reported to be stable in samples stored at $-80 \, ^\circ C$ for at least 48 h. There are some concerns that some degree of oxidation will take place during the separation of plasma, even if it is conducted as quickly as possible. With appropriate precautions, the redox status of CoQ_{10} can be estimated both by differences in electrochemical response and by differences in chromatographic behavior of ubiquinone and ubiquinol. LC–MS/MS may also be used to measure CoQ_{10} and $CoQ_{10}H_2$ simultaneously, as they are resolved chromatographically. We have observed, as reported by Hansen et al. (Hansen et al., 2004), that owing to the instantaneous oxidation of the column eluate at the ionization source, the daughter ion for the ubiquinol peak is the same as that for ubiquinone, but the chromatographic resolution is excellent and the quantitation is reliable. As yet, however, there have been no reports of clinical results of CoQ_{10} redox based on MS/MS, but there are several based on ECD.

In normal human plasma samples, the reported fraction of oxidized CoQ_{10}, expressed as the percentage of total CoQ_{10} present as ubiquinone, has varied from $6.74 \pm 0.86\%$ (Niklowitz et al., 2002) to $3.4 \pm 0.9\%$ (Sohmiya et al., 2004), with a slightly greater fraction oxidized ($4.7 \pm 1.8\%$) in patients with Parkinson disease. Ubiquinone was reported to constitute 7.9–10% of total CoQ_{10} in samples from human neonates (Menke et al., 2000).

Miles et al. (Miles et al., 2004) reported the fraction of CoQ_{10} as ubiquinone to be $2.02 \pm 2.36\%$ and $2.12 \pm 2.84\%$ in plasma samples from young children and older children, respectively, but to be significantly greater, $3.66 \pm 10.42\%$, in samples from adults.

5. Summary

The methodology for clinical measurement of CoQ_{10} is well developed; UV absorption has been used effectively for some time, but there are particular advantages to electrochemical detection and tandem mass spectrometry. Accurate quantitation of CoQ_{10} is important to discriminate disease states, and to help interpret clinical trials, many of which to date have not reported CoQ_{10} levels. At the same time, the significance of plasma CoQ_{10} concentration is not clear, and its correlation to disease and therapeutic response is often not evident. As intracellular, and intramitochondrial concentration is more significant, measurement of CoQ_{10} in platelets and nucleated blood cells should be of increased interest in the near-term future, to correlate with muscle levels. Measurement of CoQ_{10} redox state has important implications, but technical considerations regarding sample processing and analytic stability still do need to be taken into consideration.

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Mitochondrial targeting of quinones: Therapeutic implications

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Abstract

Mitochondrial oxidative damage contributes to a range of degenerative diseases. Ubiquinones have been shown to protect mitochondria from oxidative damage, but only a small proportion of externally administered ubiquinone is taken up by mitochondria. Conjugation of the lipophilic triphenylphosphonium cation to a ubiquinone moiety has produced a compound, MitoQ, which accumulates selectively into mitochondria. MitoQ passes easily through all biological membranes and, because of its positive charge, is accumulated several hundred-fold within mitochondria driven by the mitochondrial membrane potential. MitoQ protects mitochondria against oxidative damage *in vitro* and following oral delivery, and may therefore form the basis for mitochondria-protective therapies.
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Keywords: Mitochondria; Antioxidants; MitoQ

1. Introduction

Mitochondria have many roles that are central to eukaryotic cell function and survival (Scheffler, 1999; Murphy and Smith, 2000; Nicholls and Ferguson, 2002). Mitochondrial dysfunction disrupts the activity of cells, tissues and organs, and underlies a remarkably wide range of pathologies (Wallace, 1999; Leonard and Schapira, 2000a; Leonard and Schapira, 2000b; Murphy and Smith, 2000; Smeitink et al., 2001). In many cases where mitochondrial dysfunction contributes to disease, a major cause

of damage is believed to be reactive oxygen species (ROS) generated by mitochondria (Raha and Robinson, 2000; Balaban et al., 2005; Finkel, 2005). A series of mitochondrial antioxidant defences exists to intercept ROS and to minimize mitochondrial oxidative damage (Murphy and Smith, 2000), but excessive production of ROS or disruption to these antioxidant defences can lead to extensive oxidative damage to mitochondrial protein, lipid and DNA (Finkel, 2005). The proximal ROS is thought to be superoxide produced by the respiratory chain, probably at complexes I and III, which then dismutates to hydrogen peroxide (Raha and Robinson, 2000; Balaban et al., 2005). Although superoxide itself is not particularly reactive with most biomolecules (Sawyer and Valentine, 1981), it does react with aconitase to release hydrogen peroxide and ferrous iron (Vasquez-Vivar et al., 2000). In the presence of free transition metals such as ferrous iron, hydrogen peroxide generates the extremely reactive hydroxyl radical (Vasquez-Vivar et al., 2000). In addition,

superoxide reacts with nitric oxide to form the very damaging oxidant peroxynitrite (Beckman et al., 1990). These are likely to be the major ways in which mitochondrial ROS cause damage to mitochondrial protein, lipid and DNA (Sawyer and Valentine, 1981; Fridovich, 1997; Vasquez-Vivar et al., 2000). As increased mitochondrial oxidative damage is either a primary cause or a significant secondary factor leading to cell damage and death in the majority of degenerative diseases, a general therapy to decrease mitochondrial oxidative damage would be of use in a wide range of clinical situations (Murphy and Smith, 2000; Finkel, 2005; Skulachev, 2005).

Coenzyme Q_{10} and related quinones have been used as therapies to decrease mitochondrial oxidative damage (Shults et al., 2002; Shults et al., 2004). Orally administered ubiquinones can be taken up by mitochondria, but only to a limited extent (Ernster and Dallner, 1995; Matthews et al., 1998). One way to overcome this is to target ubiquinone to mitochondria by conjugation to the lipophilic triphenylphosphonium (TPP^+) cation (Murphy, 1997; Murphy and Smith, 2000; Murphy, 2001; Smith et al., 2003a; Smith et al., 2004). This procedure leads to an orally bioavailable molecule, MitoQ, which accumulates into the cell driven by the plasma membrane potential and then accumulates further into the mitochondria. When the ubiquinone moiety is reduced to its active ubiquinol redox form it can protect mitochondria against oxidative damage, after which it is recycled back to its active form by the mitochondrial respiratory chain (Fig. 1).

2. Accumulation of MitoQ by cells and mitochondria

Two features of lipophilic cations make them effective for delivering ubiquinones to mitochondria: they can pass directly through phospholipid bilayers without requiring a specific uptake mechanism, and they accumulate substantially within mitochondria due to the membrane potential (Liberman et al., 1969; Ketterer et al., 1971; Ross et al., 2005). From numerous studies we have a detailed understanding of how lipophilic cations such as triphenylmethylphosphonium ($TPMP^+$) move through phospholipid bilayers (Ketterer et al., 1971; Honig et al., 1986; Ross et al., 2005). The energy profile for the movement of a lipophilic cation through a phospholipid bilayer is derived by combining the attractive hydrophobic effect with the repulsive electrostatic forces. When added to the energy barrier at the center of the membrane this leads to potential energy wells close to each membrane surface (Ketterer et al., 1971). The binding of TPP^+ cations within this potential energy well is on the hydrophobic side of the lipid/water interface of the membrane, at about the level of the carbonyls and C2 carbons of the phospholipid fatty acyl groups (Ketterer et al., 1971; Cafiso and Hubbell, 1981; Flewelling and Hubbell, 1986; Honig et al., 1986; Ono et al., 1994). During membrane transport, the cations initially adsorb to the membrane as a monolayer in the potential energy well on the outside surface of the membrane. They then
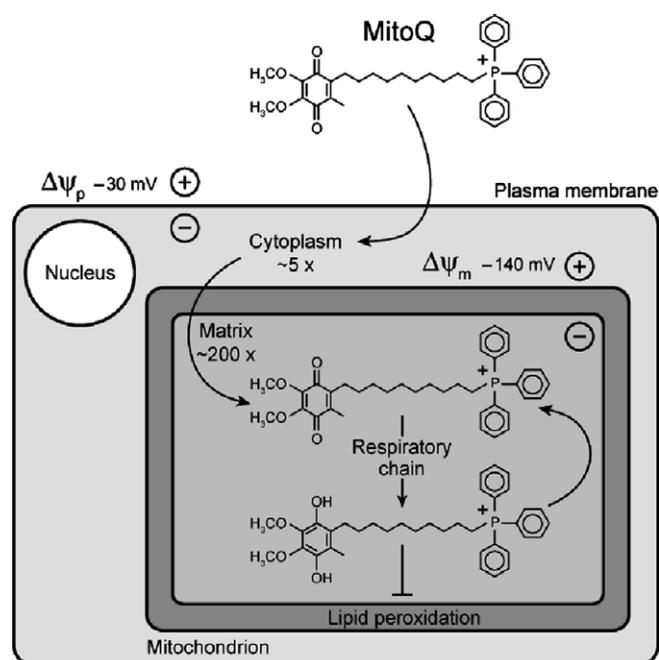
Fig. 1. Accumulation of MitoQ into cells and mitochondria. MitoQ first passes through the plasma membrane and accumulates in the cytosol driven by the plasma membrane potential ($\Delta\psi_p$). From there, MitoQ is further accumulated several hundred-fold into mitochondria driven by the mitochondrial membrane potential ($\Delta\psi_m$). MitoQ is then reduced to the active antioxidant ubiquinol form by complex II in the mitochondrial respiratory chain. Interaction with ROS produces the oxidized ubiquinone form which can subsequently be reduced again by the respiratory chain. In mitochondria, MitoQ will be mainly adsorbed to the inner surface of the mitochondrial inner membrane with the antioxidant moiety located within the hydrophobic bilayer.

pass through the hydrophobic core of the membrane to the potential energy well on the other surface of the membrane, before desorbing from the membrane.

Lipophilic cations will be taken up from a positively charged compartment into a negatively charged compartment until a sufficiently large concentration gradient is built up to equalize the electrochemical potential of the molecules in the two compartments. At this point, when the uptake of the compound has equilibrated with the membrane potential ($\Delta\psi$), the ratio of the concentrations of the free, unbound cations in the two compartments is described by the Nernst equation:

$$\Delta\psi = \frac{2.303RT}{F}\log_{10}\left(\frac{[\text{cation}_{\text{in}}]}{[\text{cation}_{\text{out}}]}\right)$$

As 2.303RT/F is 59.5–61.5 mV at physiological temperatures (25–37 °C), there will be ~10-fold accumulation of the cation for every ~60 mV increase in $\Delta\psi$. The plasma membrane potential ranges from about 30 to 60 mV (negative inside), therefore lipophilic cations will accumulate 5–10-fold into the cytoplasm. As the mitochondrial $\Delta\psi$ in cells is typically 140–180 mV (Azzone et al., 1984; Brand, 1995), the cations within the cytosol will further accumulate several hundred-fold within mitochondria, effectively

localizing a large proportion of them within mitochondria (Fig. 1).

3. Interaction of MitoQ with mitochondrial membranes

The uptake of TPP$^+$ cations by energized mitochondria was introduced over three decades ago by Skulachev and co-workers to investigate the mitochondrial $\Delta\psi$ (Bakeeva et al., 1970; Liberman and Skulachev, 1970) and has been used routinely since that time to measure $\Delta\psi$ (Azzone et al., 1984; Brand, 1995). The interaction of TPP$^+$ cations with phospholipid bilayers has also been studied extensively in order to probe the structures and electrostatics of biological membranes; consequently, a considerable amount is known about how they pass through membranes (Ketterer et al., 1971; Cafiso and Hubbell, 1981; Flewelling and Hubbell, 1986; Honig et al., 1986; Ono et al., 1994). The model for the uptake of TPP$^+$ cations through phospholipid bilayers has a number of important implications for the interaction of MitoQ with mitochondria. First, the steady state concentration of lipophilic cations in the hydrophobic core of the membrane is negligible even though they move easily through the membranes. Second, these compounds have a strong tendency to adsorb onto the surface of phospholipid bilayers in response to the local potential energy well. Third, the more hydrophobic alkylTPP$^+$ cations will have a stronger membrane adsorption tendency. Furthermore, while the TPP$^+$ component is always found at the same position in the potential energy well, namely on the membrane surface, the hydrophobic alkyl chain of MitoQ is likely to be inserted into the hydrophobic core of the membrane (Demura et al., 1985; Ono et al., 1994; Smith et al., 2004; James et al., 2005b). Finally, within energized mitochondria, the majority of the compound taken up into mitochondria is thought to be bound to the matrix-facing surface of the mitochondrial inner membrane (Kelso et al., 2001).

3.1. MitoQ in isolated mitochondria

MitoQ is taken up rapidly by isolated mitochondria driven by the $\Delta\psi$, and within mitochondria nearly all the accumulated MitoQ is adsorbed to the matrix surface of the inner membrane (Kelso et al., 2001). Inside mitochondria, MitoQ is reduced to the active ubiquinol antioxidant by complex II in the respiratory chain, but it is not a good substrate for complex I (James et al., 2005b). MitoQ can also be reduced by α-glycerophosphate dehydrogenase in mitochondrial membranes, but as the active site for this enzyme is on the outer surface of the mitochondrial inner membrane, it may not be an important MitoQ reduction site *in vivo* when the $\Delta\psi$ is substantial (James et al., 2005b). MitoQ cannot restore respiration in mitochondria lacking coenzyme Q, possibly because the reduced form of MitoQ is poorly oxidized by complex III; consequently, all the effects of MitoQ are likely to be due to the accumulation of the antioxidant ubiquinol form and not to any

effects on respiration (James et al., 2005b). Furthermore, when the ubiquinol form of MitoQ acts as an antioxidant, it is oxidized to the ubiquinone form which is then rapidly reduced again by complex II, restoring the antioxidant efficacy (James et al., 2005b). This recycling to the active form after neutralization of a ROS is a critical factor in the efficacy of many antioxidants (Ames et al., 1993; Frei et al., 1990; James et al., 2004; Sies, 1993). MitoQ is probably found adsorbed to the inner surface of the inner mitochondrial membrane with its side chain and quinone moiety penetrating into the core of the membrane. Therefore, it was anticipated to be an effective antioxidant against lipid peroxidation, and this has been confirmed for isolated mitochondria (Asin-Cayuela et al., 2004; James et al., 2005b). MitoQ has also been shown to detoxify peroxynitrite and be oxidized by superoxide or the hydroperoxyl radical, although as with other ubiquinols, its reactivity with hydrogen peroxide is negligible (James et al., 2005b; Kelso et al., 2001).

The compound initially synthesized (MitoQ) contained a 10-carbon chain linking the TPP$^+$ and ubiquinone moieties (Kelso et al., 2001). The dependence of efficacy on chain length was tested by creating and examining a series of related molecules with different numbers of carbons (3, 5, 10, 15) in the linking chain. It was found that the shorter chain analogues were less effective antioxidants than the 10-carbon compound (Asin-Cayuela et al., 2004; James et al., 2005b). This was due in part to their slower reduction to the ubiquinol form and may be a consequence of their poor access to the active sites of ubiquinone reductases in the respiratory chain (James et al., 2005b). The longer chain compound with the 15-carbon linker was very lipophilic and adhered strongly to membranes (Asin-Cayuela et al., 2004). Therefore, all of the extended studies have been carried out with the 10-carbon chain compound MitoQ.

3.2. Interaction of MitoQ with cells

Toxicity is the first issue to examine in determining whether MitoQ can act as an antioxidant in cells. It is well known that the extensive accumulation of lipophilic cations within isolated mitochondria at concentrations approaching millimolar levels can disrupt membrane integrity, respiration and ATP synthesis (Azzone et al., 1984; Bakeeva et al., 1970; Brand, 1995; Murphy, 1997). These effects are thought to be largely a result of adsorption of the lipophilic cations to the matrix surface of the inner membrane, disrupting membrane permeability and affecting enzyme and transporter activity. Supporting this idea, the more hydrophobic TPP$^+$ cations can disrupt mitochondrial function at lower concentrations and the degree of disruption correlates with the amount of compound adsorbed to the inner membrane. Thus, MitoQ starts to increase the respiration rate of isolated mitochondria when about 2.5–5 μM of the compound is added to the incubation, while 5–10-fold higher concentrations of TPMP$^+$ are required to show

the same effects (Kelso et al., 2001). The non-specific effects of MitoQ on mitochondria are assessed using the control compound decylTPP$^+$ which is similar in hydrophobicity to MitoQ (octanol/PBS partition coefficients of 5000 (James et al., 2005b) and 2760 (Asin-Cayuela et al., 2004), respectively) but lacks the antioxidant ubiquinol moiety (James et al., 2005b). We find that the non-specific cellular toxicity of MitoQ and decylTPP$^+$ occurs at similar concentrations (Ross, M.F., unpublished observations) and these non-specific effects on mitochondrial function will always limit the concentration of TPP$^+$-derived targeted antioxidants which can be used. Therefore, it is essential that the compounds are effective antioxidants at concentration levels well below those that disrupt function.

In cells, the targeted antioxidants will be accumulated into the cytosol 5–10-fold relative to the extracellular environment by the plasma membrane potential (Brand, 1995; Murphy, 1997; Nicholls and Ferguson, 2002). As a result, to achieve the same intramitochondrial concentration in cells as for studies with isolated mitochondria, it is necessary to incubate cells at lower concentrations of antioxidant. Operation at MitoQ concentrations used effectively with isolated mitochondria will often disrupt mitochondrial function within the cells. In yeast, toxicity due to mitochondrial disruption can be assessed by observing the effects on cell growth in non-fermentable medium. Under these conditions MitoQ and decylTPP$^+$ show similar toxicity with no evident effects at 0.1 μM, but toxicity is apparent at 1 μM and becomes severe at 10 μM, whereas TPMP$^+$ is far less toxic (Cochemé, H.M., unpublished observations). Short-term toxicity to mammalian cells in culture can be avoided by operating at concentrations in the range 0.1–1 μM (Ross, M.F., unpublished observations). However this varies considerably with cell density, type and incubation conditions and should be checked carefully for all new experimental arrangements. In establishing these parameters for the non-specific toxic effects of MitoQ, we find decylTPP$^+$ to be a useful routine control (James et al., 2005a), although another group uses TPP$^+$-undecanol as a control (Kalivendi et al., 2005).

3.3. Uptake and distribution of MitoQ within cells

It is well known that tetraphenylphosphonium and TPMP$^+$ can be taken up into cells through the plasma membrane (Azzone et al., 1984; Brand, 1995). The uptake of MitoQ is faster than that of TPMP$^+$, presumably due to its greater hydrophobicity effectively lowering its activation energy for passage through the plasma membrane (Kelso et al., 2001). MitoQ uptake into cells is largely blocked by abolishing the mitochondrial membrane potential with the protonophore carbonylcyanide-p-trifluoro methoxyphenylhydrazone (FCCP), consistent with uptake being primarily into the mitochondria and not to other cell compartments (Kelso et al., 2001). These data are consistent with rapid equilibration of compounds such as MitoQ across the plasma membrane, followed by their accumulation into mitochondria. However, it is technically difficult to confirm that a lipophilic cation taken up by cells is actually located within mitochondria, as during cell subfractionation the mitochondria depolarize and rapidly release MitoQ. Attempts at rapid cell subfractionation in 143B cells indicated that approximately 50% of the added MitoQ was located in the mitochondria (Kelso et al., 2001), although this figure is likely to be an underestimation due to the rapid loss of MitoQ from mitochondria upon cell homogenization. An alternative way of visualizing the location of lipophilic cations within cells is by using 4-iodobutyltriphenylphosphonium (IBTP$^+$) in which the TPP$^+$ cation is linked to an iodoalkyl system, which reacts with protein thiols to form a stable thioether linkage (Lin et al., 2002). This chemical bond prevents loss of the functionalized TPP$^+$ cation during cell fixation and the location can be visualized by using TPP$^+$-specific antiserum. The results from these experiments indicate almost total mitochondrial uptake of IBTP$^+$ within cells with very little remaining outside the mitochondria (Lin et al., 2002), suggesting that in cultured cells, nearly all accumulated lipophilic cations are present within mitochondria. However, this approach may underestimate the non-mitochondrial uptake due to the abundance of thiols within mitochondria and the higher pH in the matrix which make thiols more reactive with alkyl halides in that environment (Lin et al., 2002). One further indication of the $\Delta\psi$-dependent mitochondrial concentration of TPP$^+$-containing molecules within cells arises from the observation that while MitoQ was 800-fold more potent than idebenone in a cell model of Friedreich's ataxia, the MitoQ concentration required to afford protection was increased 25-fold in the presence of FCCP. A control experiment showed that FCCP did not affect the potency of decylQ or idebenone (Jauslin et al., 2003). This is fully consistent with MitoQ protecting against the damage in this cell model due to its $\Delta\psi$-dependent uptake into mitochondria. Therefore, strong evidence exists that upon incubation with cells in culture, MitoQ is predominantly accumulated within the mitochondria.

4. Protection against oxidative damage by MitoQ in cells

MitoQ has been used in a large range of mitochondrial and cell models, where it showed protection against various types of oxidative damage. In several cases it was shown that MitoQ decreased ROS production from mitochondria passing into the cytoplasm, as assessed by measuring the fluorescence of dichlorofluorescein (Hwang et al., 2001; Saretzki et al., 2003; Schafer et al., 2003; Barhoumi et al., 2004; Dhanasekaran et al., 2004; King et al., 2004; Kalivendi et al., 2005; Koopman et al., 2005; Pletjushkina et al., 2005; Siler-Marsiglio et al., 2005). An increase in cytosolic dichlorofluorescein fluorescence is generally interpreted as resulting from increased hydrogen peroxide production, suggesting that MitoQ decreases the efflux of hydrogen peroxide from mitochondria. However, the mechanism of this is unclear as MitoQ does not react directly with hydrogen

peroxide (James et al., 2005b), so MitoQ may be acting upstream of mitochondrial hydrogen peroxide production. Alternatively, it could be that the ROS which reacts with dichlorofluorescein is not actually hydrogen peroxide at all but some other compound, such as peroxynitrite or a lipid peroxidation breakdown product. The interaction of MitoQ with mitochondrial ROS production within rotenone-treated fibroblasts has been studied in detail (Koopman et al., 2005). MitoQ did not decrease superoxide production as measured by hydroethidine oxidation but it did prevent mitochondrial lipid peroxidation as determined by the fluorescent probe C11-BODIPY (Pap et al., 1999; Koopman et al., 2005). These findings are consistent with the model for MitoQ action developed from studies with isolated mitochondria, namely that the main antioxidant function of MitoQ is to prevent mitochondrial lipid peroxidation. It remains to be seen if this is the major mechanism by which MitoQ acts as a protective agent in all cell types and forms of oxidative stress.

There are a number of other mechanisms by which MitoQ could potentially affect mitochondrial ROS metabolism. ROS production is thought to require a high $\Delta\psi$, therefore the accumulation of lipophilic cations in mitochondria could slightly uncouple mitochondria, decreasing the $\Delta\psi$ and thus lowering ROS production by reverse electron transport (Lambert and Brand, 2004a; Lambert and Brand, 2004b). In isolated mitochondria, both MitoQ and decylTPP$^+$ can decrease mitochondrial ROS production by this mechanism (James, A.M., unpublished observations), however this remains to be explored in cells. Another possibility is that MitoQ may act as a pro-oxidant generating superoxide (James et al., 2004). All ubiquinols, including that derived from MitoQ, can deprotonate ($pK_a = 11.3$) in water to form the ubiquinolate anion which facilitates the formation of superoxide from oxygen (James et al., 2004). However, this superoxide production pathway is diminished if deprotonation of the ubiquinol moiety is prevented by maintaining it in the lipid phase (James et al., 2004). Consistent with this, MitoQ produces less superoxide by autoxidation than more hydrophilic versions in the presence of mitochondrial membranes (James et al., 2005b). In yeast mitochondria, superoxide produced by MitoQ is too low to cause damage to mitochondrial aconitase (James et al., 2005b), and in fibroblasts, MitoQ does not increase superoxide production (Koopman et al., 2005). Even so, it has been postulated that such a non-damaging flux of ROS could increase expression of antioxidant defence enzymes and thus increase protection, a process called hormesis (Calabrese et al., 2006). Future work will examine how antioxidant defences respond to the long-term presence of MitoQ. To summarize, MitoQ is protective against oxidative stress in a range of cell systems exposed to various forms of oxidative stress. Current evidence indicates that MitoQ acts predominantly as an antioxidant by preventing lipid peroxidation of the mitochondrial inner membrane. However, more experimentation is required to determine if this model applies to all cell types and forms of oxidative stress and the possibility remains that some effects of MitoQ may involve non-antioxidant mechanistic pathways.

5. Administration and distribution of MitoQ *in vivo*

While it is clear that MitoQ is a useful research tool in isolated mitochondria and cells, to function as a therapy the compound must be delivered to mitochondria within cells in patients, preferably following oral administration. As TPP$^+$ cations pass easily through phospholipid bilayers, they should be able to cross from the gut to the bloodstream and from there reach most tissues, including the brain through the blood–brain barrier. It has been shown that the TPMP$^+$ cation can be taken up into mitochondria within a perfused heart (Kauppinen, 1983; Wan et al., 1993), liver (Steen et al., 1993) and skeletal muscle (Rolfe and Brand, 1996a; Rolfe and Brand, 1996b; Rolfe et al., 1999), driven by the mitochondrial $\Delta\psi$. Then TPMP$^+$ and other simple alkylTPP$^+$ compounds were administered to mice by intravenous injection, they were rapidly distributed to the heart, brain, skeletal muscle and other organs (Srivastava et al., 1985; Fukuda et al., 1986; Smith et al., 2003b). In contrast, intraperitoneal administration of TPMP$^+$ to mice resulted in most of the compound going initially to the liver, with some later redistribution to the heart but very little to the brain (Smith et al., 2003b). Intravenous administration of MitoQ to mice showed that these compounds were rapidly cleared from the plasma post-administration and were also taken up into the same tissues as noted for TPMP$^+$ (Smith et al., 2003b). These experiments clearly indicate that, once in the bloodstream, alkylTPP$^+$ compounds are rapidly cleared into organs, consistent with their accumulation into mitochondria. However, as was the case with cells, it is difficult to be certain of the whereabouts of the compound within the cell due to their rapid redistribution from the mitochondria upon homogenizing the tissue. One surrogate approach is to use the probe IBTP$^+$ (Lin et al., 2002). Four hours after IBTP$^+$ had been given to mice by intravenous injection, there was accumulation into the heart mitochondria, but not the cytosol. This is consistent with predominantly mitochondrial localization of TPP$^+$ cations from the bloodstream (Smith et al., 2003b).

An important advance in this area was the observation that TPP$^+$-derived compounds were orally bioavailable to mice. This was achieved by feeding mice [^{3}H]-TPMP$^+$ in their drinking water which resulted in uptake into the plasma and from there into the heart, brain, liver and muscle (Smith et al., 2003b). Similarly, after several days of feeding 500 μM MitoQ in drinking water, there was significant uptake into heart, liver, kidney, brain and white adipose tissue (Smith et al., 2003b). When administration was stopped, the alkylTPP$^+$ compounds were cleared from the tissues. These studies are consistent with orally administered alkylTPP$^+$ compounds distributing to all organs due to their facile permeation through biological

membranes, and subsequently re-equilibrating back into the plasma when administration was terminated.

The non-specific toxicity of alkylTPP$^+$ cations that is found with mitochondria and cells will also occur *in vivo* and this will probably be the major factor limiting the amount of the compound which can be administered safely. In crude toxicity assessments with mice (Smith et al., 2003b), it was found that a single intravenous dose of MitoQ was well tolerated at 750 nmol ($\sim$20 mg/kg) with toxicity evident at 1000 nmol ($\sim$27 mg/kg). Administering 500 μM of MitoQ in the drinking water could be maintained for several weeks. No toxic effects were noted in mice fed 500 μM MitoQ in liquid diet for at least 26 days or in drinking water for at least 14 days (Smith et al., 2003b). While the published research outlined above has been relatively limited and served as a proof of principle, more formal toxicity studies have also been undertaken in developing MitoQ for clinical trials and these are outlined below under the pharmaceutical development of MitoQ.

5.1. Efficacy of MitoQ in vivo

As discussed above, it is possible to administer mitochondria-targeted ubiquinones long-term to animals and obtain accumulation of the compounds in the heart, brain and skeletal muscle without toxicity. The next step is to determine whether the amount of accumulated MitoQ is sufficient to act as an antioxidant *in vivo*. To date only a few trials of this nature have been carried out. When 500 μM MitoQ was administered to rats in their drinking water for two weeks and the hearts then isolated and exposed to ischemia-reperfusion injury in a Langendorff perfusion system, there was protection against the loss of heart function, tissue damage and mitochondrial function compared with controls (Adlam et al., 2005). The accumulation of MitoQ in the hearts was 20 ± 9 pmol/g wet weight, which corresponds to an intramitochondrial concentration of 100–200 nM (Adlam et al., 2005). No significant protection was observed with TPMP$^+$ nor with a short chain hydroxyalkylquinone, indicating that the effect of MitoQ was due to its accumulation into tissue mitochondria and protection against mitochondrial oxidative damage, rather than a simple consequence of lipophilic cation accumulation or an increase in cytoplasmic antioxidant activity. The most probable antioxidant mechanism for the observed protection by MitoQ in these experiments is that lipid peroxidation in the mitochondrial inner membrane was being prevented (Paradies et al., 2004). However, this has yet to be confirmed by measuring markers of oxidative damage in the mitochondria and by demonstrating that MitoQ can block this damage.

6. Pharmaceutical development of MitoQ

The development of MitoQ as a pharmaceutical is somewhat different from that of most other pharmaceuticals.

Typically, in medicinal chemistry a large number of compounds based on a lead compound that interacts with a specific target, such as a receptor binding site, are investigated. In assessing these compounds, the 'rule of five' is often used as a preliminary screen to ensure that the compounds selected have the physicochemical properties to make good drug candidates (Lipinski et al., 1997). This rule favours compounds that are soluble, bioavailable and can pass through phospholipid bilayers as judged by the values of their molecular weight, partition coefficient and number of protonatable groups. However, mitochondria-targeted antioxidants based on TPP$^+$ lipophilic cations are less constrained by these traditional guidelines since they have the unusual property of being both very water-soluble and membrane-permeant. Even though the molar mass of the MitoQ methanesulfonate salt is relatively high for a pharmaceutical and it has a high octanol/PBS partition coefficient (Asin-Cayuela et al., 2004), it is readily bioavailable and passes easily through biological membranes. A further unusual feature of the TPP$^+$-targeted compounds is that they are targeted to an organelle to modify a general rather than a specific process, namely oxidative damage. An important additional feature of MitoQ is that it can be recycled to its active antioxidant form by the action of complex II in the mitochondrial respiratory chain (James et al., 2005b). Therefore, if lipophilic cations such as MitoQ prove to be effective pharmaceuticals, it represents an unusual approach to medicinal chemistry and pharmaceutical development.

MitoQ has now been developed as a pharmaceutical (http://www.antipodeanpharma.com/). To obtain a commercially satisfactory stable formulation, it was found beneficial to synthesize the compound as the methanesulfonate salt and to further inhibit decomposition on storage by complexation with a solid support such as β-cyclodextrin. This preparation was readily made into tablets and has passed through conventional animal toxicity tests with no observable adverse effect at 10.6 mg/kg. The oral bioavailability was determined to be about 10% and the major metabolites in urine are glucuronides and sulfates of the reduced hydroquinone form along with demethylated derivatives. In phase I trials, MitoQ showed good pharmacokinetic behavior with oral dosing at 1 mg/kg resulting in a plasma $C_{max} = 33.15$ ng/ml and $T_{max} \sim 1$ h. This formulation has good pharmaceutical characteristics, and phase II trials against Parkinson's disease are now in progress, with trials for Friedreich's ataxia planned in the near future (http://www.antipodeanpharma.com/). Therefore, experience to date suggests that it should be possible to develop a range of mitochondria-targeted compounds based on the TPP$^+$ cation as orally available pharmaceuticals for the treatment of a large number of disorders.

7. Conclusions

The use of MitoQ to increase the antioxidant defences of mitochondria has been shown to be a viable strategy

in vitro. It has also been shown that MitoQ can be formulated into a pharmaceutical, which can be successfully delivered orally to humans. Animal experiments have shown that MitoQ displays some antioxidant efficacy in tissues and therefore the scene is set for testing this and related compounds in human diseases. It will be important to ascertain definitively whether MitoQ is acting as an effective antioxidant *in vivo* and whether by so doing it improves the outcome of the disease pathology. An intriguing aspect of the use of mitochondria-targeted antioxidants is that they could, in principle, be applied to a range of diseases and organs, as mitochondrial oxidative damage contributes to so many disorders. In addition they could be applied to acute injuries such as ischemia-reperfusion injury during surgery, to semi-acute situations such as liver damage from steatohepatitis, and to chronic degenerative diseases such as Parkinson's disease, Friedreich's ataxia or type II diabetes. It may even be possible to administer some of these compounds prophylactically. Hopefully work over the next few years will indicate in which organs these compounds are effective, whether they can decrease mitochondrial oxidative damage in diseases, and whether this positively affects the outcome for the patient. Additionally, it will be useful to determine whether these compounds are best applied to acute, mid-term or long-term interventions.

Acknowledgements

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Coenzyme Q, oxidative stress and aging

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Abstract

Coenzyme Q (CoQ) has three well-characterized functions in mitochondria, namely (i) transfer of reducing equivalents in the electron transport chain, (ii) generation of superoxide anion radical, $O_2^{\cdot-}$, and (iii) quenching of free radicals. The main purpose of this review is to discuss the effects of CoQ_{10} intake for relatively prolonged periods on mitochondrial respiratory capacity, indicators of oxidative stress, and life span of animals, in context of the broader issue of whether or not the overall progression of the aging process can be modified by CoQ_{10} administration. Comparative studies on different mammalian species have indicated that the rate of mitochondrial superoxide anion radical generation is directly correlated with mitochondrial CoQ_9 content and inversely related to amounts of CoQ_{10}, particularly the CoQ_{10} bound to mitochondrial membrane proteins. Contrary to the historical view, dietary supplementation of mice and rats with CoQ_{10} has been demonstrated to augment the endogenous CoQ content ($CoQ_9 + CoQ_{10}$) in mitochondria and homogenates of various tissues, albeit to varying extent. Ingestion of CoQ_{10} results in the elevation of endogenous CoQ_9, the predominant homologue in mice and rats. In our studies, there was no indication of a discernable effect of CoQ_{10} intake reflecting enhancement of mitochondrial respiratory activity, antioxidant capacity and pro-oxidant potentiation or prolongation of life span. The possibility that CoQ_{10} intake affects certain other biological functions by as yet unelucidated mechanisms cannot be ruled out as CoQ has been shown to broadly alter gene expression in mice.
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1. Introduction

Coenzyme Q (CoQ) or ubiquinone (2,3-dimethoxy-5-methyl-6-multiprenyl-1,4-benzoquinone) molecules are located in the hydrophobic domain of the phospholipid bilayer of cellular membranes (Battino et al., 1990; Lenaz et al., 1999). CoQ is composed of a tyrosine-derived quinone ring, linked to a polyisoprenoid side chain, consisting of 9 or 10 subunits in higher invertebrates and mammals (Ernster and Dallner, 1995). The benzoquinone ring can assume three alternate redox states: the fully oxidized or ubiquinone (Q); the univalently reduced ($1e^- + 1H^+$) ubisemiquinone ($\bullet$QH), a free radical; and the fully reduced

($2e^- + 2H^+$) ubiquinol. The polyisoprenyl chain apparently facilitates the stability of the molecule within the hydrophobic lipid bilayer. In addition, the length of the CoQ isoprenoid chain seems to affect the mobility, intermolecular interaction with membrane proteins, and autoxidizability (Matsura et al., 1992; James et al., 2004). The physiological roles of CoQ in biological systems are most well characterized in the inner mitochondrial membrane, where three of its main functions are: (i) carrier of electrons from respiratory complexes I and II to complex III, (ii) generation of superoxide anion radical by autoxidation of ubisemiquinone and (iii) anti-oxidant quenching of free radicals (Crane and Navas, 1997; James et al., 2004; Turunen et al., 2004). The apparently paradoxical property of mitochondrial CoQ to potentially act both as a pro-oxidant and an antioxidant would seem to suggest that it may

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also be a modulator of the cellular redox state under physiological and/or pathological conditions, and particularly it may play a role in the aging process.

It is now widely recognized that during aging there is a pro-oxidizing shift in the cellular redox state, accompanied by an accrual of oxidatively damaged molecules, which in combination may play a causal role in senescence (Sohal and Weindruch, 1996; Sohal et al., 2002). The main premise of this hypothesis, often referred to as the oxidative stress hypothesis, is that the imbalance between pro-oxidant generation and anti-oxidant defenses, or the level of oxidant load or stress, increases during aging, and mitochondria play a critical role in this homeostatic perturbation. The reason why mitochondria are thought to be implicated is that the rates of mitochondrial $O_2^{\cdot-}/H_2O_2$ production and oxidative damage to mitochondrial DNA, proteins and lipids increase during aging (Sohal and Sohal, 1991; Sohal et al., 1994a,b; Sohal and Dubey, 1994). An important consequence of this apparently self-initiated damage is the acceleration of the rate of reactive oxygen species (ROS) generation, a phenomenon first reported in (Sohal and Sohal, 1991; Sohal and Dubey, 1994).

Another major age-related mitochondrial alteration is that ADP-stimulated (state 3) and the maximal (uncoupled) rates of mitochondrial oxygen consumption decline during aging (Ferguson et al., 2005). These twin changes, i.e., elevation of oxidative stress/damage due to enhanced $O_2^{\cdot-}/H_2O_2$ production, and the decline in mitochondrial ability to synthesize ATP, are thought to progressively attenuate the functional capacity of various physiological systems. CoQ is suspected to be involved in both of these age-related alterations because as an electron carrier, CoQ is a component of the oxidative phosphorylation system, and because CoQ is also an ROS generator and a quencher. The main question though is whether there is a cause-and-effect relationship between age-related changes in mitochondria and the CoQ-related parameters. In this context, the main purpose of this review is to examine whether variations in CoQ content or the relative abundance of its homologues affect mitochondrial function and have an impact on the aging process.

2. Effect of age on CoQ content in different tissues

CoQ content as well as the ratios of Q_9 and Q_{10} vary in different organelles, tissues and species. For instance, lysosomes and Golgi membranes generally contain relatively higher concentrations of CoQ than mitochondrial membranes or microsomes (Dallner and Sindelar, 2000). In mice, the variation in CoQ content among homogenates of different tissues is about 100-fold, with rank order: kidney > heart > skeleton > muscle > brain > liver (Lass et al., 1999a; Lass and Sohal, 2000). Compared to the homogenates, CoQ ($CoQ_9 + CoQ_{10}$) content of mitochondria was greater by six-times in liver, three-times in kidney, four-times in heart and 23-times in the skeletal muscle. CoQ concentrations in mitochondria also varied in different tissues, with heart containing 3.6-, 3.3-, 2.7- and 1.5-times higher amounts than those in the kidney, liver, brain, and skeletal muscle, respectively.

Results of the various studies in the literature on the age-related changes in CoQ levels do not support the existence of a common trend. For instance, Kalen et al. (1989) reported an age-related loss of CoQ content in homogenates of human tissues. Beyer et al. (1985) found no age-related changes in CoQ level in homogenates of rat brain and lungs, an increase in the liver, and a decrease in the heart, kidney and skeletal muscles. Battino et al. (1995) reported a decline in CoQ content in nonsynaptic mitochondria from mouse brain between 2 and 18 months of age, followed by an increase at 24 months of age.

Studies in this laboratory suggest that age-associated changes in CoQ content are most evident in mitochondria, which also preferentially sequester CoQ, rather than in the homogenate. For instance, in the rat, there were no significant differences in the amounts of CoQ_9 or CoQ_{10} in the plasma or the tissue homogenates of liver, heart and kidney at 4-, 19-, or 24 months of age (Kamzalov and Sohal, 2004). In contrast, mitochondrial content of CoQ_9, which is the predominant homologue, declined with age in all three tissues. A previous study showed a similar loss in mitochondria of rat skeletal muscle (Lass et al., 1999b). Homogenates of mouse liver, heart, kidney, skeletal muscle and brain also showed no age-related loss in CoQ_9 or CoQ_{10} content (Sohal et al., 2006). Rather, during the period of 7–19 months of age, CoQ_9 content increased in brain and skeletal muscle, respectively, by 20% and 12%, while CoQ_{10} content increased 33% and 52%. The aforementioned discrepancies between the findings from different laboratories may be due to the specific ages of the sampled animals or the procedure used for extraction and quantification of CoQ, or differences in species/strains/diets.

To summarize, it seems that during aging, decreases in CoQ content may occur in mitochondria of some tissues in certain species, but such losses are selective rather than ubiquitous. It has also been experimentally shown that physiological concentrations of CoQ in mitochondria do not exceed those required for kinetic saturation of NADH-Q-oxidoreductase, suggesting that CoQ is rate limiting in the electron transport chain (Estornell et al., 1992). Therefore, decreases of CoQ below the physiological levels can potentially affect mitochondrial respiratory function, which may indeed occur under specific pathological conditions.

3. Inter-species variations in mitochondrial CoQ content and superoxide anion radical generation

The variations observed in the amounts of CoQ and ratios of the CoQ homologues among different tissues raise the question whether they carry any physiological significance, particularly whether differential amounts of CoQ_9 or CoQ_{10} affect mitochondrial functions. This issue was

addressed by us by first comparing the concentrations of CoQ homologues in cardiac mitochondria from nine different mammalian species, namely mouse, rat, guinea pig, rabbit, goat, sheep, pig, cow and horse (Lass et al., 1997; Sohal et al., 1999). The total amount of CoQ ($CoQ_9 + CoQ_{10}$) present in different species varied about 2-fold with the rank order: horse = mouse = cow = sheep = goat > rat > pig = rabbit > guinea pig; however, the ratios of CoQ_{10}:CoQ_9 in different species varied 60-fold. The total CoQ content was not found to be correlated with maximum life span (MLS) of the species. In contrast, CoQ_9 content was inversely and CoQ_{10} directly correlated with the MLS of the species (Fig. 1). For instance, CoQ_9 concentration was 40 to 60-times greater in mitochondria of shorter-lived species such as mouse or rat compared to the longer-lived horse or cow.

In separate studies involving comparisons of different mammalian species (Sohal et al., 1989, 1999; Ku et al., 1993), we found that species-specific maximum life span was inversely correlated with mitochondrial rates of $O_2^{\cdot-}$
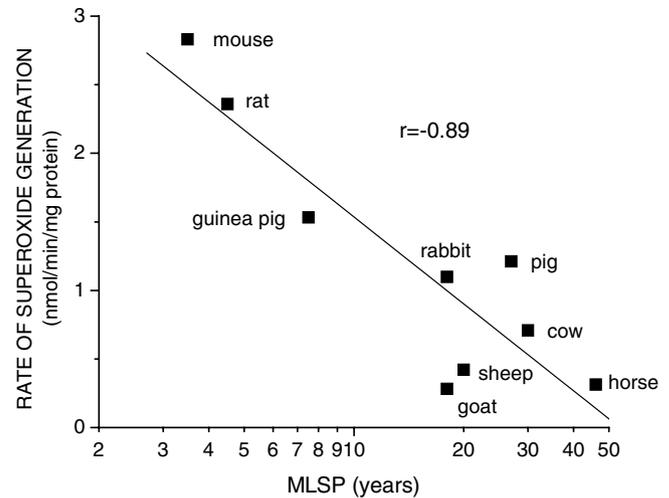
Fig. 2. Relationship between rate of superoxide anion radical generation by cardiac submitochondrial particles and maximum life span potential (MLSP) of different species, expressed as $\log_{10}$ years. (Adapted from data in Lass et al., 1997).
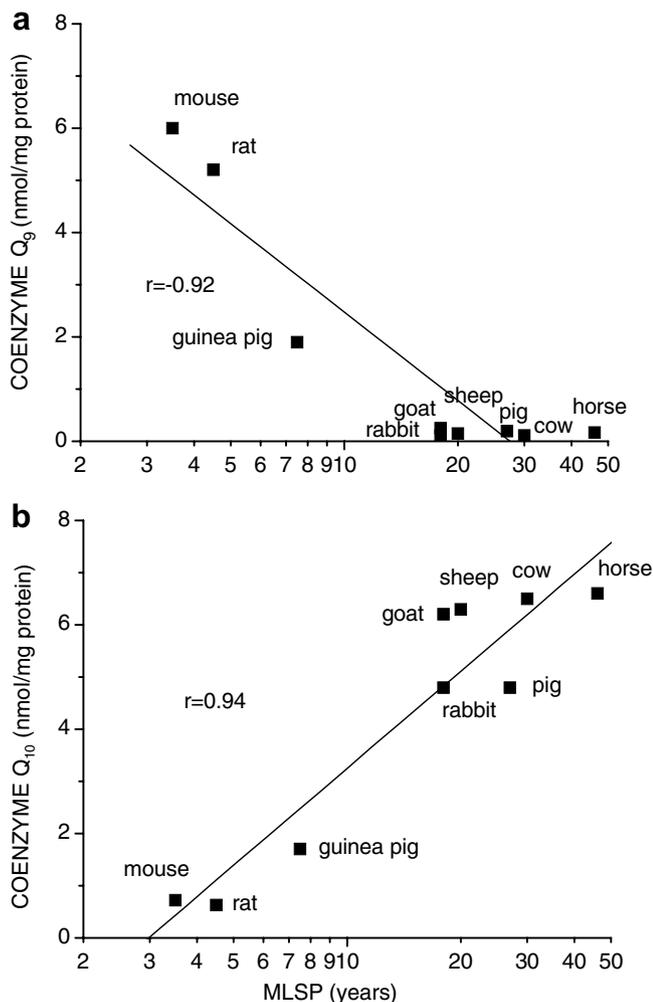
and H_2O_2 generation (Fig. 2). It was thus of interest to further examine whether CoQ_9 or CoQ_{10} content or the ratio of the two were also correlated with the rate of mitochondrial $O_2^{\cdot-}$ generation (Lass et al., 1997; Sohal et al., 1999). CoQ_9 content of cardiac mitochondria was found to correlate directly and CoQ_{10} inversely with the rate of $O_2^{\cdot-}$ generation (Fig. 3).

Such correlations led to the hypothesis that CoQ_9 content of mitochondria might be a determinant of the rate of $O_2^{\cdot-}$ generation. This possibility was addressed in CoQ depletion/repletion studies on submitochondrial particles (SMPs) of rat and cow, which differ 16-fold in total CoQ content (Fig. 4). Depletion of CoQ, followed by repletion with different amounts of CoQ_9 or CoQ_{10}, indicated that at relatively lower concentrations, there were no significant differences in $O_2^{\cdot-}$ generation between repletions with equal amounts of CoQ_9 or CoQ_{10} (Lass et al., 1997). However, at repletions with relatively higher concentrations of CoQ_9 or CoQ_{10}, the rates of $O_2^{\cdot-}$ generations were greater in CoQ_9- than CoQ_{10}-augmented SMPs. This can be interpreted to suggest that increased CoQ_9 content may lead to relatively higher rates of $O_2^{\cdot-}$ generation *in vivo*. Interestingly, an increase in the rate of $O_2^{\cdot-}$ generation due to CoQ_9 augmentation of SMPs of rat was an order of magnitude higher than that in cow SMPs, suggesting that structural organization of the inner mitochondrial membrane also plays a determining role in the rate of $O_2^{\cdot-}$ production.

To further examine the possibility that binding of CoQ and mitochondrial membrane proteins (presumably the oxidoreductases of the electron transport chain), modulates the rate of $O_2^{\cdot-}$ generation, proteins in mitochondrial membranes were isolated as micelles from five different mammalian species that varied in their relative amounts of CoQ_9 and CoQ_{10} (Lass and Sohal, 1999). It was found that up to 32% of the total mitochondrial CoQ is bound to proteins, the rest presumably belonged to the freely dif-

Fig. 1. Relationship between CoQ_9 (a) and CoQ_{10} (b) content of heart mitochondria from different species and maximum lifespan potential (MLSP) of the species, expressed as $\log_{10}$ years. (Adapted from data in Lass et al., 1997).
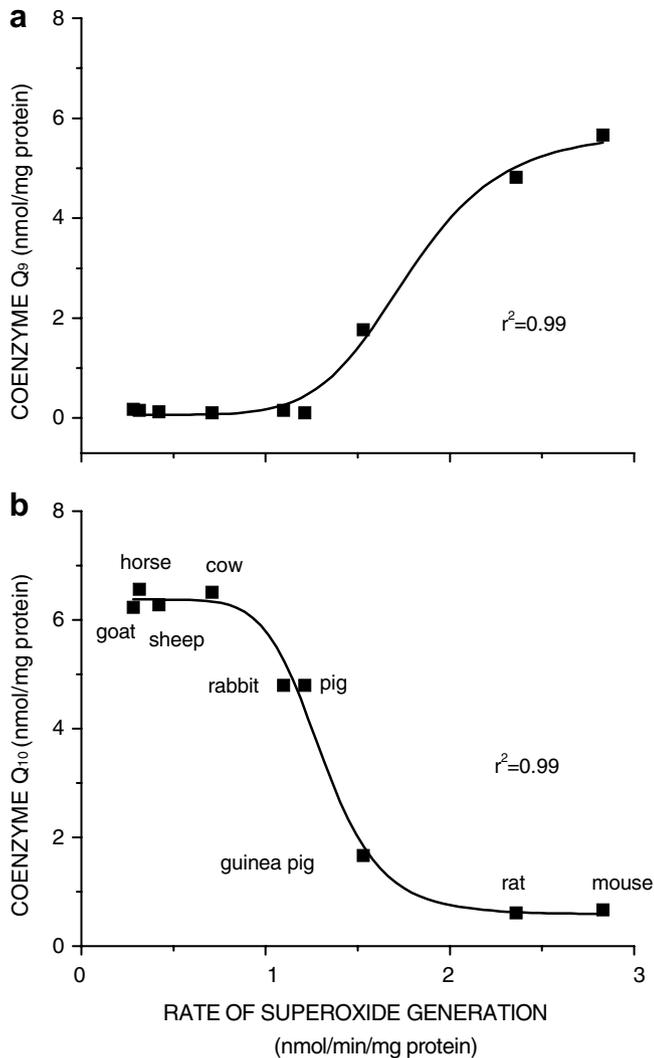
Fig. 3. Relationship between rates of superoxide anion radical generation by cardiac submitochondrial particles from different species and amounts of CoQ$_9$ (a) and CoQ$_{10}$ (b) extracted from heart mitochondria. (From Lass et al., 1997).

Fig. 4. Rates of superoxide anion radical generation by CoQ-depleted/repleted rat (a) and bovine (b) heart submitochondrial particles (SMPs). Freeze-dried SMPs were depleted of native CoQ by six repeated extractions with pentane and reconstituted with specific amounts of CoQ homologues, followed by measurements of rates of O$_2^{\cdot-}$ generation. (From Lass et al., 1997).

fusible pool. Notably, the amount of protein-bound CoQ in micelles of mitochondria from heart of different species was found to be inversely related to the rate of mitochondrial O$_2^{\cdot-}$ generation (Fig. 5). For instance, micelles from cow and pig contained twofold higher amounts of CoQ than those from rat and mouse. It is worth pointing out that those micelles with relatively high CoQ:protein ratio were derived from species that are longer-lived and have CoQ$_{10}$-rich mitochondria with relatively low rates of O$_2^{\cdot-}$ generation. On the basis of such studies, it can be hypothesized that variations in longevity among different species co-evolved with the increases in the amounts of CoQ$_{10}$ bound to the mitochondrial proteins.

Altogether, results of the various correlational studies discussed above suggest that variations in the relative amounts of CoQ homologues may be associated with rates of mitochondrial O$_2^{\cdot-}$ generation as well as longevity of different species; notwithstanding, such associations do not establish a cause-and-effect relationship. Experimental tests of this idea would require manipulations of the relative amounts of CoQ homologues.

4. Effect of CoQ administration on endogenous CoQ content

CoQ is synthesized endogenously by the mevalonate pathway (Ernster and Dallner, 1995). The historical view about the contribution of the dietary sources to endogenous levels of CoQ (Reahal and Wigglesworth, 1992; Zhang et al., 1995, 1996), reiterated recently (Bentinger et al., 2003), is that although ~6% of the orally administered CoQ permeates the gastrointestinal tract into the blood and is transferred to liver and spleen, CoQ uptake by other tissues such as heart, kidney, brain and skeletal muscle is low or completely absent, unless the endogenous levels have fallen below a critical threshold. More recent studies, however, have reported that this historical perspec-
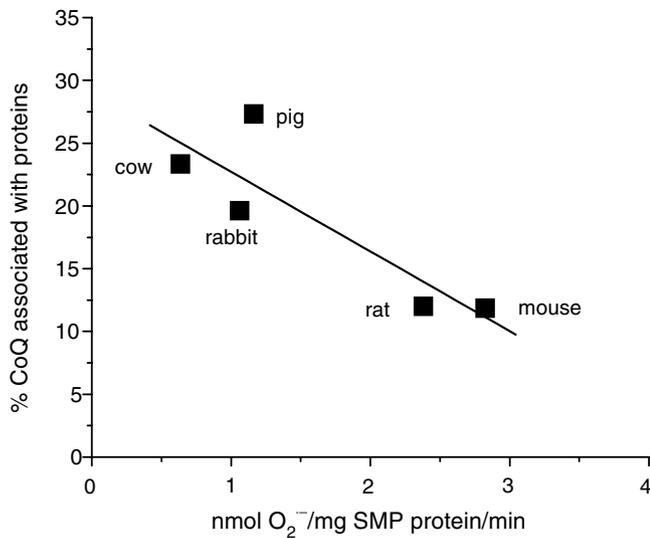
Fig. 5. Relationship between amounts of CoQ associated with membrane proteins and rate of superoxide anion radical generation by cardiac mitochondria of different mammalian species. Mitochondrial membrane proteins were isolated as deoxycholic acid (DOC) pelletable micelles, and the CoQ_9 and CoQ_{10} content was determined by HPLC. The CoQ content $(CoQ_9 + CoQ_{10})$, retained in the mitochondrial DOC micelles in different species, is expressed as percent of the total amount of CoQ present in whole mitochondria and is plotted against the rate of $O_2^{\cdot-}$ generation, reported previously by Lass et al. (1997).

tive was based on administration of relatively low dosages of CoQ for a comparatively short period. It was first shown by Matthews et al. (1998) that CoQ_{10} intake by 12- or 24-month-old rats increased CoQ content in brain mitochondria and had a neuroprotective effect against 3-nitropropionic acid. Subsequently, a series of studies conducted by us (Lass et al., 1999a,b; Lass and Sohal, 2000; Kwong et al., 2002; Kamzalov et al., 2003; Rebrin and Sohal, 2004) demonstrated that CoQ_{10} administration via food to young adult mice or rats caused an increase in amounts of both CoQ_9 and CoQ_{10} homologues in plasma, and in homogenates and mitochondria of liver, heart and skeletal muscle (Fig. 6). In the brain, the increase was of a lesser magnitude and occurred primarily in mitochondria and not the homogenate. In all the tissues, the amount of CoQ augmentation was greater in mitochondria than in the homogenate, suggesting its preferential sequestration in mitochondria. These studies also indicated that CoQ_{10} administration enhanced endogenous CoQ_9, by a mechanism that remains to be elucidated. In general, greater augmentations of CoQ $(CoQ_9 + Co_{10})$ could be achieved with increases in the duration of the CoQ_{10} administration. These augmentations were equivalent to or greater than those achieved by short-term increases in CoQ dosage. Furthermore, when compared with the relatively hydro-
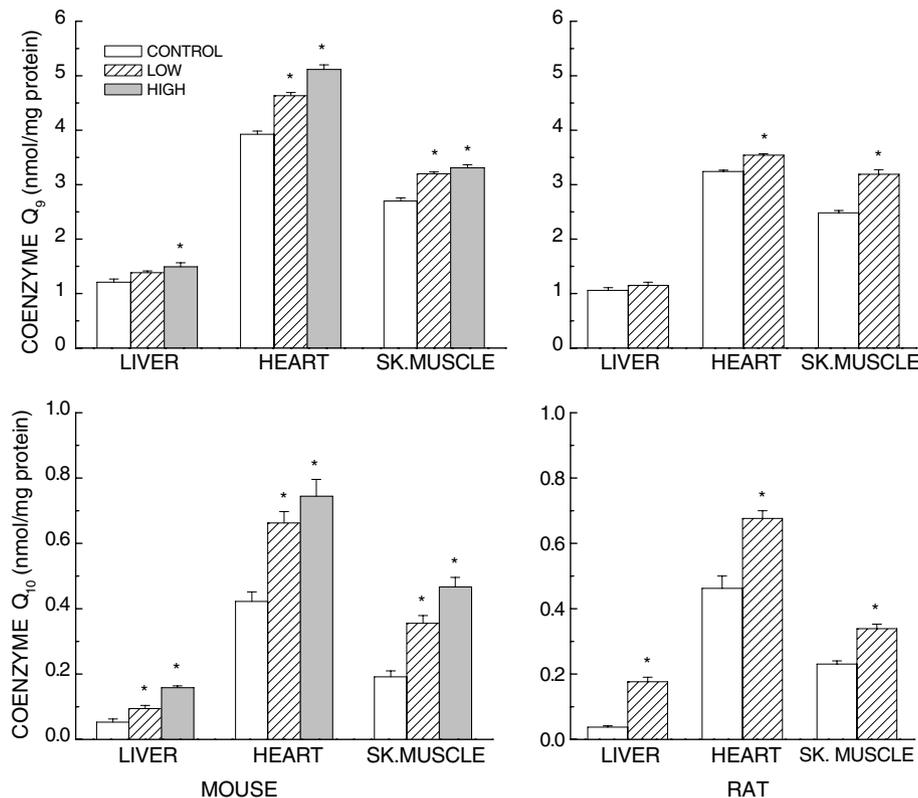
Fig. 6. Effect of CoQ_{10} intake on CoQ_9 (top panels) and CoQ_{10} (bottom) content in mitochondria from different tissues of young adult mice (left) or rats (right). In studies on mice (Kamzalov et al., 2003), CoQ_{10} was added to the food yielding daily CoQ intakes of 148 or 654 mg/kg body weight for 11 weeks. Rats were fed an amount equivalent to the low dose (150 mg/kg/d) for 13 weeks prior to tissue collection (Kwong et al., 2002). Values are means $\pm$ SE for 5–8 mice or 6–7 rats; $*p < 0.05$ when compared with the control group (planned individual comparison within one-way ANOVA). The mouse data are from Kamzalov et al. (2003).

phobic CoQ powder, a water-miscible CoQ_{10} formulation (Q-Gel from Tishcon) resulted in more consistent augmentation of endogenous CoQ, particularly in brain (see Kwong et al., 2002; Kamzalov et al., 2003 versus Lass et al., 1999a; Sohal et al., 2006). Notwithstanding, it should be noted that the uptake of CoQ and other lipoidal substances is a complex process dependent upon a number of different factors.

5. Antioxidant roles of CoQ and α-tocopherol in inner mitochondrial membrane

The inner mitochondrial membrane contains CoQ as well as α-tocopherol, both of which have antioxidant properties, thereby raising the issue about their respective roles in quenching the free radicals generated in inner mitochondrial membrane. In solutions, CoQ has been shown to inhibit lipid peroxidation in mitochondrial membranes that have been depleted of α-tocopherol (Mellors and Tappell, 1966; Takayanagi et al., 1980). α-Tocopherol has also been unambiguously demonstrated to be capable of scavenging lipid peroxyl radicals, thereby preventing the propagation of chain reactions during lipid peroxidation (McCay, 1985). Notwithstanding, the reactivity of α-tocopherol with peroxyl radical, which generates α-tocopheroxyl radical, far exceeds that of peroxyl radicals with ubiquinol, thereby suggesting that ubiquinol is unlikely to be a direct radical scavenger *in vivo*. Current evidence suggests that ubiquinol and α-tocopherol act in concert to scavenge radicals during autoxidation of mitochondrial membranes (Kagan et al., 1990; Stoyanovsky et al., 1995; Lass and Sohal, 1998, 2000; Sohal, 2004). α-Tocopherol seems to act as a direct scavenger forming tocopheroxyl radical, whereas ubiquinol reacts with tocopheroxyl radical to regenerate α-tocopherol. Several studies seem to confirm the sparing/regenera- tive effect of CoQ on α-tocopherol *in vivo* (reviewed in Sohal, 2004). In young adult mice, CoQ_{10} intake effectively augmented the α-tocopherol concentration in tissue homogenates and mitochondria from liver, heart, and skeletal muscle (Kamzalov et al., 2003). A similar effect was observed in homogenates and mitochondria of rats (Fig. 7).

6. CoQ intake, mitochondrial function and life span in mice

Because CoQ can act as a generator or a quencher of ROS and because it is widely consumed by humans as a dietary supplement motivated us to conduct studies on the effects of long-term intake of CoQ_{10} on the aging process. Specifically, we examined whether CoQ_{10} intake affects the level of oxidative stress, mitochondrial respiratory functions, or the survival of the animals (Sohal et al., 2006). Mice were fed diets providing daily supplements of 0, 93, or 371 mg CoQ_{10}/kg body weight, starting at 3.5 months of age. Amounts of CoQ_9 and CoQ_{10}, determined at 7 and 21 months of age, were found to increase in relation to dosage and duration of CoQ_{10} administration. Augmentations of CoQ were detected in homogenates and mitochondria of liver, heart, kidney, and skeletal muscle, and were evidently maintained throughout life. Different tissues tended to vary in their capacity for CoQ accretion, with liver and skeletal muscle exhibiting the highest elevations, and the brain showing the least. Thus, these studies confirmed our previous observations in young adult mice and rats, firmly refuting the long-held notion that CoQ content of tissues other than plasma, liver or spleen cannot be significantly augmented by dietary administration of CoQ_{10}. Indeed these demonstrations have provided the necessary rationale for testing the effects of CoQ_{10} intake on a broad range of parameters associated
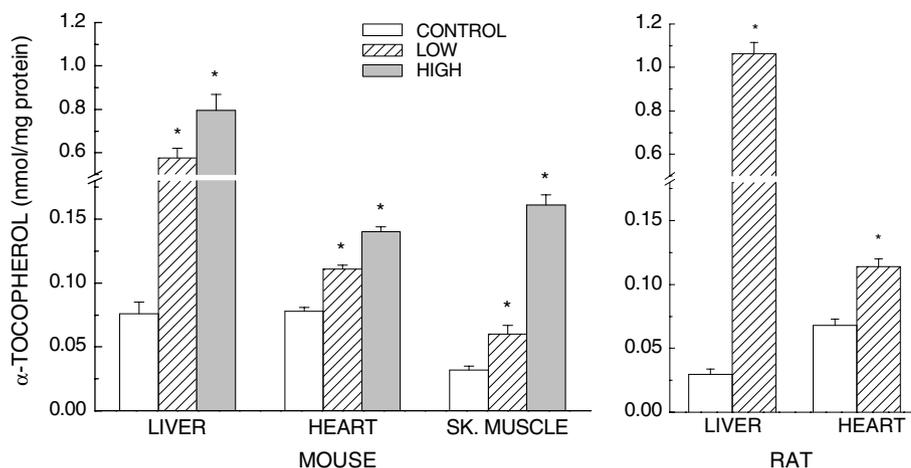
Fig. 7. Effect of coenzyme Q_{10} intake on α-tocopherol concentration in mitochondria from different tissues of young adult mice or rats. In studies on mice (Kamzalov et al., 2003), CoQ_{10} was added to the food in different amounts yielding daily CoQ intakes of 148 (low) or 654 (high) mg/kg body weight for 11 weeks. Rats consumed an amount equivalent to the low dose (150 mg/kg/d) for 13 weeks prior to tissue collection (Kwong et al., 2002). Values are means ± SE for 5–8 mice or 6–7 rats; *$p < 0.05$ when compared with the control group (planned individual comparison within one-way ANOVA). Data for mouse are from Kamzalov et al. (2003); α-tocopherol concentrations in rat mitochondria are from previously unpublished data from the study described by Kwong et al. (2002).

with mitochondrial functions, oxidative stress, and life span.

Long-term CoQ_{10} intake, lasting from 3.5 up to 25 months had no effect on activities of the major antioxidant enzymes, such as catalase, glutathione peroxidase and superoxide dismutase in liver, kidney, skeletal muscle or brain. Similarly, long-term CoQ_{10} intake had no effect on mitochondrial respiratory chain, measured as activities of oxidoreductases such as NADH ferricytochrome c reductase (complex I/III) and ferrocytochrome c oxidase (complex IV). The rates of oxygen consumption by liver mitochondria, measured as glutamate/malate and succinate supported state-3 respiration, were also not affected by CoQ_{10} intake. To further determine whether CoQ_{10} intake affected the level of oxidative stress, rates of $O_2^{\cdot-}$ generation were measured in SMPs from heart, kidney and skeletal muscle of 25-month-old experimental and control mice. No significant differences were detected between the groups. Levels of protein carbonyls and GSH:GSSG ratios also showed no notable effect of CoQ_{10} intake. Thus, prolonged CoQ_{10} intake seemingly failed to modulate mitochondrial respiratory capacity or levels of oxidative stress.

CoQ_{10} administration, starting at 3.5 months of age, also had no significant effect on the long-term survival of the mice (Fig. 8). Two previous studies on mice and rats in other laboratories, in which CoQ_{10} was administered at dosages lower than the highest dosage used by us, also reported no effect on life span (Lonnrot et al., 1998; Lee et al., 2004). Results of studies in other species are, however, quite contradictory and species-specific. For instance, different groups have reported that life span of the worm, *Caenorhabditis elegans*, is prolonged by CoQ_{10} supplementation (Asencio et al., 2003), or, paradoxically, by a deficiency of CoQ in the diet (Larsen and Clarke, 2002) or

Fig. 8. Survival plots for groups of 50 mice fed a control diet or low or high amounts of CoQ_{10}. The low (0.72 mg/g of food) and high (2.81 mg/g) CoQ diets were introduced when the mice were 3.5 months of age and yielded daily CoQ intakes of approximately 93 or 371 mg/kg body weight throughout life. Survivorship, expressed as Kaplan–Meier probability, was not significantly different from the control for the mice with low or high intakes of CoQ ($p > 0.065$, Tarone-Ware). Adapted from Sohal et al. (2006).

even by the inability of a mutant strain to synthesize the normal CoQ homologue (Branicky et al., 2000). One explanation of these contradictory findings may be the unusual life history and the mode of adaptation of *C. elegans* to stress. A deficiency of CoQ induces a hypometabolic or a dauer-like state, which in nature facilitates survival under adverse conditions. For instance, the life span of the worm is extended by the intake of antimycin A, an inhibitor of the electron transport chain, which is extremely toxic to most other aerobic species (Dillin et al., 2002). Notwithstanding, the results of studies on the effects of CoQ_{10} intake on the life span of mammals are in agreement that CoQ_{10} supplementation has no effect on longevity.

7. CoQ intake and brain function in mice

Concurrently with our studies of long-term intake of CoQ_{10} on the aging process in mice, we also examined the effect of supplementation of CoQ_{10} alone, α-tocopherol alone or CoQ_{10} + α-tocopherol on cognitive and motor functions of 24-month-old mice, which had been treated for 13 weeks (McDonald et al., 2005). CoQ_{10} intake alone increased α-tocopherol concentrations in plasma, liver, heart, and skeletal muscle of these 24-month-old mice, however, in the brain tissue of old mice, the mitochondrial α-tocopherol concentration was augmented only when CoQ_{10} and α-tocopherol were administered together. This treatment (CoQ_{10} + α-tocopherol) also partially ameliorated impaired learning of an avoidance problem by these old mice, whereas treatments with CoQ_{10} alone or α-tocopherol alone were relatively less effective. None of the treatments improved age-related impairment of the mice in tests of motor performance. In a follow-up experiment, much higher doses of CoQ_{10} administration also failed to improve avoidance performance of the old mice. These studies suggested that intake of α-tocopherol and CoQ_{10} together has a synergistic effect.

To conclude, although CoQ may act as a pro-oxidant or an antioxidant *in vitro*, it has no notable *in vivo* effects on mitochondrial respiratory functions or levels of oxidative stress. There is also no clear indication of its ability to influence the life span or brain function of mammals, however, it is conceivable that beneficial effects of CoQ_{10} intake may occur under certain pathological conditions. It is also worth noting that CoQ_{10} intake has been shown to broadly affect the pattern of gene expression (Lee et al., 2004; Groneberg et al., 2005), indicated by the abundance of mRNAs, however, the nature of the physiological impact remains to be demonstrated.

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Infantile and pediatric quinone deficiency diseases

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Abstract

Coenzyme Q_{10} (CoQ_{10}) plays a pivotal role in oxidative phosphorylation (OXPHOS) as it distributes electrons between the various dehydrogenases and the cytochrome segments of the respiratory chain. Primary coenzyme Q_{10} deficiency is a rare, but possibly treatable, autosomal recessive condition with four major clinical presentations, an encephalomyopathic form, a generalized infantile variant with severe encephalopathy and renal disease, a myopathic form and an ataxic form. The diagnosis of ubiquinone deficiency is supported by respiratory chain analysis and eventually by the quantification of CoQ_{10} in patient tissues. We review here the infantile and pediatric quinone deficiency diseases as well as the clinical improvement after oral CoQ_{10} therapy. The clinical heterogeneity of ubiquinone deficiency is suggestive of a genetic heterogeneity that should be related to the large number of enzymes, and corresponding genes, involved in ubiquinone biosynthesis.
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Keywords: Ubiquinone; Coenzyme Q_{10}; Mitochondria

1. Introduction

Coenzyme Q (CoQ, ubiquinone) is a lipophilic component located in the inner mitochondrial membrane that has a pivotal role in oxidative phosphorylation (OXPHOS). Indeed, CoQ shuttles electrons from complex I and complex II to complex III. Being in large excess compared to any other component of the respiratory chain (RC), it forms a kinetically compartmentalized pool, the redox status of which tightly regulates the activity of the dehydrogenases. CoQ also plays a critical function in anti-oxidant defenses. Ubiquinone is composed by a redox active benzoquinone ring connected to a long isoprenoid side chain. Ubiquinone is present in prokaryotes and eukaryotes but the length of its isoprenoid chain varies among species. Humans and rodents produce mainly CoQ_{10} and CoQ_9, respectively, whereas *Saccharomyces cerevisiae* synthesizes CoQ_6 and *Escherichia coli* CoQ_8. In human, CoQ_{10} is present in all tissues and cells but in variable amount. It ranges between 114 µg/g in heart to 8 µg/g in lung (Turunen et al., 2004). However, a small amount of CoQ_9 (2–7%) is also synthesized in human tissues. Moreover, it has been shown in bovine brain that the concentration of ubiquinone varies among cells and regions of a specific organ.

2. Clinical presentation

Primary coenzyme Q_{10} deficiency is a rare, clinically heterogeneous disorder of the respiratory chain. The first patient was described 17 years ago and less than 40 patients have been further reported. CoQ_{10} deficiency is presumably inherited as an autosomal recessive trait as all enzymes involved in ubiquinone biosynthesis pathway are nuclearly encoded.

Four major phenotypes have been described (i) a encephalomyopathic form characterized by exercise intolerance, mitochondrial myopathy, myoglobinuria, epilepsy and ataxia; (ii) a generalized infantile variant with severe encephalopathy and renal disease; (iii) a myopathic form with exercise intolerance, myoglobinuria, and myopathy;

and (iv) an ataxic form, dominated by ataxia, seizures and either cerebellar atrophy or anomalies of the basal ganglia.

2.1. Encephalomyopathic form

The first reported CoQ_{10} deficiencies were observed in two sisters with encephalomyopathy (Ogasahara et al., 1989). They were born from unrelated parents and had a normal development in the first two years of life. The onset symptoms, fatigability and muscle weakness, appeared at 3 years of age. The clinical course then included growth retardation, learning disability, seizure, dysarthria, ataxia and myopathy. Both patients had hyperlactatemia and elevation of serum creatine kinase level. Several years later, three other studies reported four additional patients (Boitier et al., 1998; Sobreira et al., 1997; Van Maldergem et al., 2002). The clinical features of all these patients including those from the first report are presented in Table 1. Two patients had a very similar clinical presentation to the first report (Boitier et al., 1998; Sobreira et al., 1997). The disease appeared before 3 years of age and the onset symptoms were muscle weakness or ataxia. The patients also presented myopathic features with or without myoglobinuria and high level of serum creatine kinase. The two more recently reported sisters (Van Maldergem et al., 2002) displayed bilateral T2 hypersignal intensity in the caudate and the putamen suggestive of Leigh syndrome. They did not have myopathy but severe mental retardation. Hearing loss and ophthalmologic symptoms were observed in 4/6 patients. All patients presented hyperlactatemia and/or hyperlactatorachia. Muscle histology was suggestive of a mitochondrial dysfunction in all patients but those with Leigh syndrome (Van Maldergem et al., 2002). Respiratory chain analysis performed on muscle always revealed normal activities of all five complexes; however quinone dependent activities (complex I + III and complex II + III) were significantly decreased. The amount of ubiquinone was consistently and deeply decreased in muscle of all patients.

2.2. Multisystemic form

A more generalized and infantile variant with severe encephalopathy and visceral organ involvement was described in four different reports (Leshinsky-Silver et al., 2003; Quinzii et al., 2006; Rahman et al., 2001; Rotig et al., 2000; Salviati et al., 2005). A total of 7 children from 4 different families harbored this multisystemic form of ubiquinone deficiency (Table 2). The patients presented a severe neurological involvement with ataxia, hypotonia, seizures, pyramidal syndrome or Leigh syndrome. Most of them (6/7) also had severe renal involvement such as nephrotic syndrome or tubulopathy requiring kidney transplant for two of them. Only one patient had a major liver involvement and presented pancreatic insufficiency. Ophthalmologic involvement or hearing loss was frequently observed. Two of these patients died (Rahman et al.,

2001; Rotig et al., 2000) whereas all other patients with ubiquinone deficiency were still live at the time of publication. Muscle histology did not revealed mitochondrial abnormalities. However, respiratory chain study performed on muscle or liver showed abnormal quinone-dependent activities. In all cases, complex I + III and complex II + III deficient activities were activated by exogenous quinone. This deficiency was also observed in fibroblasts or lymphocytes suggesting a widespread defect of ubiquinone. The quinone content was considerably decreased in fibroblasts and/or muscle of most of these patients.

2.3. Myopathic form

The myopathic form reported in six patients always associates myopathy, muscle weakness and hyperlactatemia and/or hyperlactatorachia (Auré et al., 2004; Di Giovanni et al., 2001; Horvath et al., 2006; Lalani et al., 2003). The age of onset of the disease in the myopathic form is relatively higher than in other quinone deficiency as it varies between 3 and 14 years of age (Table 3). The onset symptom is most often exercise intolerance. All patients presented elevated creatine kinase but only two had myoglobinuria. 2/5 patients presented extramuscular symptoms such as seizures, cerebellar syndrome or diabetes mellitus. Respiratory chain analysis performed in muscle revealed a deficiency of quinone dependent activities. Finally a reduced amount of ubiquinone was observed in muscle.

2.4. Ataxic form

The ataxic form seems to be the most common form of ubiquinone deficiency. This clinical presentation was discovered by chance as one of 20 "disease control" muscle samples used in the study of Sobreira et al., 1997 showed very low amount of CoQ_{10}. This patient presented with hereditary ataxia of unknown origin. This prompted the authors to investigate CoQ_{10} amount in muscle of a series of patients with unexplained ataxia and to report six similar cases (Musumeci et al., 2001). Surprisingly, three of these patients were subsequently reported to have homozygous nonsense mutation in the APTX gene, encoding aprataxin, responsible of ataxia-oculomotor apraxia (AOA1) (Quinzii et al., 2005). Aprataxin is a nuclear protein with a role in DNA repair (Moreira et al., 2001) and it could be hypothesized that the quinone deficiency has occurred independently from aprataxin mutation in this case. Later on, Lamperti reported a series of 13 patients (Lamperti et al., 2003). The last report described a Spanish patient with isolated ataxia (Artuch et al., 2006). The age of onset of the disease is relatively wide (2 months–6 years, Table 4). Ataxia was sometimes associated with other manifestations such as mental retardation (6/19), muscle weakness (6/19), seizures (6/19). All patients presented cerebellar atrophy. Lactic acidosis is seldom present and respiratory chain deficiency was suggestive of quinone deficiency in few of the

Table 1
Encephalomyopathic form of ubiquinone deficiency

Sex	Ogasahara et al. (1989)		Sobreira et al. (1997)	Boitier et al. (1998)	Van Maldergem et al. (2002)	
	♀	♀	♀	♂	♀	♀
Family history	Multiplex		Sporadic	Sporadic	Multiplex	
Age of onset	3 years	3 years	30 months	2 years	6 months	1 months
Age at publication (years)	14	12	35	3	29	31
Onset symptom	Fatigability, muscle weakness of trunkal and proximal muscles	Fatigability, muscle weakness of trunkal and proximal muscles	Delayed motor milestones	Cerebellar symptoms, ataxia, proximal motor weakness	Trunk hypotonia, failure to thrive	Diarrhea, trunk hypotonia, failure to thrive
Cerebellar ataxia	+		−	+	+	+
Other neurological involvement	Cerebellar syndrome			Cerebellar syndrome dysarthria	Pyramidal syndrome, mental retardation, regression	Dystonia, spasticity, mental retardation, regression
Growth retardation	+	+		+	+	+
Myoglobinuria	+	+	+			
Creatine kinase	×20	×20	×4	×23		
Learning disability	+	+		Delayed language development	+	+
Seizures	Abnormal EEG	+	+	+		
Myopathy	+	+	Muscle pain after exercise	+	+	+
Weakness	+	+	+	+	+	
Ptosis			+	+	+	
Additional features			Migraine headache, vomiting,tilted optic disk, mild scoliosis	Hypotrophy, retinitis pigmentosa	Dysmorphic features, deafness, no puberty	Deafness, growth delay, facial dysmorphy
MRI/CT scan	nd	nd	Cerebral atrophy	Severe cerebellar atrophy, no leukodystrophy	Bilateral T2 hyperintensity signal in the caudate and putamen	
Hyperlactatemia/ hyperlactatorachia	+	+	Mild	+	+	+
Muscle histology	Mitochondrial and lipid excess	Mitochondrial and lipid excess	RRF	RRF, atypical lipid droplets	Fiber type disproportion	Selective hypotrophy of type 2 fibers
RC deficiency	M: I + III, II + III		M: I + III II + III	M: I + III II + III	M: II + III	
Q content	M: 67 ng/mg prot (N = 1811 ± 99)	M: 98 ng/mg prot (N = 1811 ± 99)	M: 5.6 nmol/g tissue (N = 69 ± 23)	M: 114 µg/g tissue (N = 1856 ± 112)	M: 43 µg/g prot (N = 793)	M: 493 µg/g prot (N = 793)
Q content (%)	M: 3.7%	M: 5.4%	M: 8%	M: 16%	M: 5%	M: 40%
Q supplementation	150 mg/d	150 mg/d	150 mg/d	60-250 mg/d	300 mg/d	300 mg/d
Improvement after Q supplementation	+	+	+	Less weak, normalized CPK, no neurological improvement	Decreased hypertonia, recover ability to walk, weight gain, improved social interactions	Growth parameter, behavior

M, muscle; RRF, ragged red fibers; N, normal; nd, not done.

Table 2
Multisystemic form of ubiquinone deficiency

Sex	Rotig et al. (2000)			Rahman et al. (2001)	Leshinsky et al. (2003)	Salviati et al. (2005); Quinzii et al. (2005)	
	♂	♀	♀	♂	♂	♂	♀
Family history	Multiplex				Consanguineous	Multiplex, consanguineous	
Age of onset	Few weeks	6 months	1 yr	6 h	5 days	2 months	
Age at publication	16 years	17 years	Death at 8 years	Death at 2 years	2 months	33 months	9 months
Onset symptom	Nystagmus, severe myopia	Delayed motor development	Deafness	Poor feeding, hypothermia	Neurological distress	Nystagmus	
Liver involvement					Cholestatic jaundice		
Renal involvement	Nephrotic syndrome, kidney transplantation	Nephrotic syndrome, glomerular sclerosis	Nephrotic syndrome, glomerular sclerosis, kidney transplantation	Tubulopathy		Severe nephrotic syndrome, focal and segmental glomerulosclerosis	Nephropathy
Ophthalmologic involvement	Retinitis pigmentosa, left optic nerve atrophy, right cataract, myopia	Visual loss	Myopia, nystagus			Optic atrophy, retinopathy	
Neurological involvement	Ataxia, dystonia, hypotonia			Seizures	Leigh syndrome	Hypotonia, seizures, psychomotor regression	
Deafness	+	+	+				
Ataxia	+	+	+				
Amyotrophy	+						
Heart involvement	Hypertrophic cardiomyopathy			Left ventricular hypertrophy			
Other signs	Dystonia	Pyramidal syndrome			Pancreatic insufficiency		
Delay	Mental retardation		Mental retardation	Developmental delay		Psychomotor delay, weakness	
MRI/CT scan		Generalized hypodensity of white matter		Cerebral atrophy, cerebellar atrophy	nd	Cerebral atrophy, cerebellar atrophy	
Hyperlactatemia/ hyperlactatorachia	N			+	+	N	
Muscle histology	nd	nd		Type 2 fiber atrophy, excess of lipid	ND	N	
RC deficiency	M, Fb, Ly: I + III II + III	M, Fb, Ly: I + III II + III		M: I + III	Li: I + III	M: I + III Fb: II + III	Fb: II + III
Q content	Fb: 0	Fb: 0		M: 37 pmol/mg prot (N = 140−580)	nd	M: 12 mg/g tissue (N=32 ± 12) Fb: 19 ng/ mg prot (N = 105 ± 14)	Fb: 18 ng/mg prot (N = 105 ± 14)
Q content (%)	Fb: 0%	Fb: 0%				M: 37%, Fb: 18%	
Q supplementation	90 mg/d	90 mg/d	nd	300 mg/d	At 2 months	30 mg/kg/d	
Improvement after Q supplementation	Ability to walk, functional improvement, cognitive improvement, cataract resolved	+		Normalized lactatemia, renal tubular function improved, but developmental delay, dystonia and seizures	Improvement of liver function, but developmental delay and deafness after 6 months of treatment	Increased muscle strength, myoclonus disappeared, but no improvement of renal function	

M, muscle; Fb, fibroblasts; Ly, lymphocytes; Li, liver; RRF, ragged red fibers; N, normal; nd, not done.

Table 3
Myopathic form of ubiquinone deficiency

Sex	Di Giovanni et al. (2001)	Auré et al. (2004)	Lalani et al. (2003)	Horvath et al., (2006)	
	♂	♀	♂	♀	♂
Family history	Multiplex	Sporadic	Sporadic	Sporadic	Sporadic
Age of onset (years)	12	3	11	14	6
Age at publication (years)	15	11	11.5	33	7
Onset symptom	Exercise intolerance	Exercise fatigability, vomiting	Progressive muscle weakness	Diabetes mellitus, hypertension	Muscle hypotonia
Myopathy	+	+	+	+	+
Muscle weakness	+	+	+	+	+
Exercise intolerance	+	+			
Myoglobinuria	+		−		
Creatine kinase	×37	×20	+	×10	×8
Other signs	Seizures	Cerebellar syndrome, seizures			
MRI	N	Cerebellar atrophy, vermis atrophy	N	N	nd
Hyperlactatemia/ hyperlactatorachia	+	+	+	+	+
Muscle histology	RRF COX- fibers	Mitochondrial myopathy	RRF		
RC deficiency	M: I + III II + III	M: I + III II + III	M: I + III II + III		
Q content	M: 27 nmol/g tissue (N = 69 ± 23)	M <1 µg/g (N = 19−30)		M: 0.6 mmol/unit CS (N = 2.7−7)	M: 1.7 mmol/unit CS
Q content (%)	M 39%		M: 46%		
Q supplementation	200–300 mg/d	6 mg/kg/d (5 years)	150–300 mg/d	500 mg/d	200 mg/d
Improvement after Q supplementation	Muscle weakness resolved, normalized CK and lactate, normalized CoQ10 concentration in muscle, lipid storage in muscle disappeared	Better exersize tolerance, normalized carnitine, but progressive cerebellar syndrome	Improvement of muscle strength, normalized lactatemia, normalized CK	Normalized CK, improvement of muscle strength	Improvement of weakness, decreased CK

M, muscle; CS, citrate synthase; RRF, ragged red fibers; N; normal; nd, not done.

Table 4
Ataxic form of ubiquinone deficiency

Sex	Musumeci et al. (2001)					Lamperti et al. (2003)	Artuch et al. (2006)
	♂	♀	♂	♀	♂	8/13 ♀, 5/13 ♂	♂
Family history	Sporadic	Sporadic	Multiplex, homozygous APTX mutation			12/13 multiplex	Father: clumsiness, frequent falls and tremor in the early years of life
Age of onset	1 year	First months	2 years	4 years	4 years	Birth-9 years	15 months
Age at publication	11 years	17 years	25 years	24 years	24 years	8–30 years	12 years
Onset symptom	Clumsy and fall frequently	Developmental delay	Clumsy, hyperactive	Clumsy, fainting spell	Drooling, poor coordination, hyperactivity	5/13: hypotonia and motor delay, 6/13: ataxia2/13: seizures	Clumsy and fall frequently
Ataxia	+	+	+	+	+	13/13	+
Cerebellar atrophy	+	Vermis atrophy	+	+	+	13/13	+
Seizures	+	+		+		4/13	
Motor delay		+				7/13	
Mental retardation		+	+			4/13	
Weakness			+			5/13	
Other signs	Dandy–Walker variant	Agenesis of the corpus callosum atrophy of the cerebellar vermis, dysphagia	Scoliosis	Scoliosis	Scoliosis, choreic movements	Myoclonus, hemiplegia, spastic paraparesis, pyramidal signs	Dysarthria
Hyperlactatemia/ hyperlactatorachia	+	N	N	N	N	1/13	N
Muscle histology	COX-def fibers	N	nd	nd	nd	nd	Subsarcolemic mitochondrial accumulation
RC deficiency	M: III	M: I	M: I + III + IV			3/13: M: I + III, II + III	M: I + III II + IIIFb: I + III II + III
Q content	M: 8.9 µg/g N = 23 ± 3.5	M: 6.6	M: 7.1	M: 7.1	M: 8.2	M: 2.9−14.8 µg/g N=27.64 ± 4.43	M: 56 nmol/g prot N = 157−488
Q content (%)	M: 35% Fb: 63%	M: 26% Fb: 38%	M: 28% Fb: 61%	M: 28%	M: 32%	M: 10-53%	
Q supplementation	600 mg/d	300 mg/d	300–3000 mg/d	300–3000 mg/d	300–3000 mg/d	200–900 mg/d	2500 mg/d
Improvement after Q supplementation	Able to walk independently but seizure worsened	Improvement but persistence of ataxia	Improvement of muscle strength independent in daily activities	Seizure disappeared but no improvement of ataxia	Can walk and climb stair	11/13 improved	Improvement of ataxia, cerebellar signs disappeared

M, muscle; Fb, fibroblasts; CS, citrate synthase; RRF, ragged red fibers; N, normal; nd, not done.

patients. However, CoQ_{10} residual amount in muscle of the patients was 21–45% of control values. For few patients was the CoQ_{10} amount also reduced in cultured skin fibroblasts.

3. Diagnosis of ubiquinone deficiency

Most of the patients (except those with the ataxic form) presented clinical symptoms and metabolic anomalies suggestive of a mitochondrial respiratory chain deficiency. Therefore, the diagnosis of CoQ_{10} deficiency is usually done by polarographic and/or spectrophotometric analysis of the respiratory chain. In these patients, the various respiratory chain enzymes are normal but the quinone-dependent activities (CI+III, CII+III) are deficient. The hypothesis of ubiquinone deficiency can be further supported by the stimulation of respiratory chain activities by exogenous ubiquinone (decylubiquinone, DQ). We observed such stimulation on fibroblasts of one of our patients with severe quinone deficiency (Rotig et al., 2000). Indeed, adding decylubiquinone stimulated whole cell respiration, succinate oxidation of permeabilized fibroblasts and succinate cytochrome c reductase activity (Fig. 1). Stimulation of respiratory chain activities by exogenous quinone has been reported in several cases (Boitier et al., 1998; Leshinsky-Silver et al., 2003; Rahman et al., 2001; Salviati et al., 2005; Sobreira et al., 1997; Van Maldergem et al., 2002).

Direct evidence of quinone deficiency is eventually given by quantification of CoQ_{10} in patient tissues. CoQ_{10} is extracted from muscle or fibroblasts with lipid solvents and the amount is determined by reverse-phase HPLC (Turunen et al., 2004).

As for the vast majority of mitochondrial diseases, ubiquinone deficiency is most often tissue specific. Indeed, whereas ubiquinone is a ubiquitous component of the respiratory chain, CoQ_{10} defects are often restricted to few tissues. This is exemplified by the clinical presentation of some patients who displayed isolated ataxia for example (Lamperti et al., 2003; Musumeci et al., 2001). Moreover, the respiratory chain deficiency can be restricted to muscle only as well as the reduced CoQ_{10} content. A few patients presented with reduced amount of CoQ_{10} in fibroblasts (Rotig et al., 2000; Salviati et al., 2005). Therefore, one should be cautious to preserve muscle samples of patients suspected of CoQ_{10} deficiency, based on enzymological data, for subsequent ubiquinone measurement.

Quinone dependent activities of the respiratory chain of patients with CoQ_{10} deficiency are often only slightly decreased despite profound CoQ_{10} depletion. Indeed, we observed low succinate cytochrome c reductase activity in fibroblasts of one of our patients (19 nmol/min/mg prot, $N = 22$–47, Fig. 1) whereas CoQ_{10} was undetectable in these cells. Similar results were also observed by Ogasahara et al., 1989. One can hypothesize that the defective cells retain a low but significant residual CoQ_{10} amount sufficient for maintaining electron transfer to complex III. However, glycerol-3P and dihydroorotate cytochrome c reductase activities have been found especially sensitive to CoQ_{10} depletion (Rotig et al., 2000). The location of these dehydrogenases on the outer surface of the inner mitochondrial membrane and/or the low affinity to CoQ_{10} may account for a higher dependency towards the ubiquinone pool. Finally, it can not be excluded that the deficient enzyme of the ubiquinone biosynthesis pathway still allows
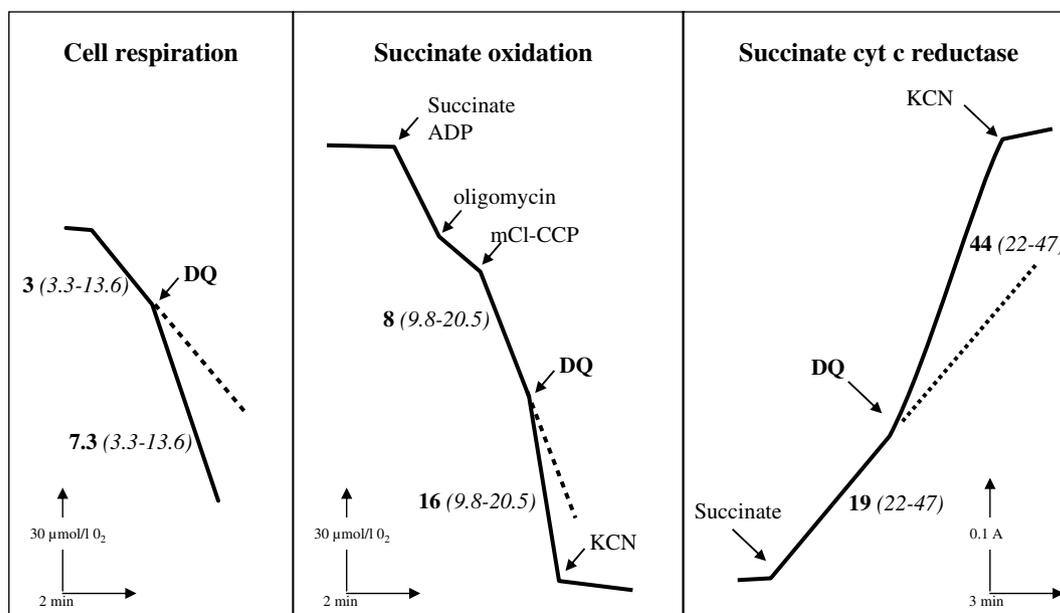

Fig. 1. Effect of an exogenous quinone analogue (decylubiquinone, DQ, 80 μM) on whole cell respiration, succinate oxidation and succinate cytochrome c reductase activity of cultured skin fibroblasts. Numbers along the traces are nmol/min/mg protein. Numbers between brackets are normal values.

production of a synthesis intermediary allowing for minimal electron transfer in the respiratory chain.

4. Replacement therapy of CoQ_{10} depletion

CoQ_{10} has been given for long to a large number of individuals with or without ubiquinone deficiency and was found to be safe. This could be related to the fact that CoQ_{10} is not taken up by the cells with normal ubiquinone content as there is no possibility to place more lipids into the limited space in the mitochondrial membrane. However, when the lipid is missing, as it is the case in ubiquinone synthesis defects, the inner mitochondrial membrane has the capacity to accept exogenous CoQ_{10} that can restore the electron flow (Turunen et al., 2004). Nevertheless, it cannot be excluded that exogenous CoQ_{10} also acts as an antioxidant allowing improvement of the general condition of some patients.

Several authors have reported a positive effect of CoQ_{10} supplementation. Patients were given various doses of ubiquinone ranging from 90 to 2000 mg/d. The small number of patients obviously precluded any statistical analysis but subjective improvement was noticed by parents and family members. Improvement could be observed after 2 months of treatment and consisted of better coordination (Van Maldergem et al., 2002), recovery of walking and bicycle riding (Rotig et al., 2000), decreased muscle weakness (Boitier et al., 1998), disappearance of myoclonus (Salviati et al., 2005), better speech articulation and improved posture and gait (Lamperti et al., 2003), and a decreased score on the ICARS scale evaluation (Artuch et al., 2006). Creatine kinase returned to normal in several cases (Di Giovanni et al., 2001; Horvath et al., 2006). Nevertheless, after a first improvement in some patients during several months or few years, additional symptoms appeared or worsened such as cerebellar atrophy and myoclonic jerks (Auré et al., 2004), developmental delay and seizures (Rahman et al., 2001), or deafness (Leshinsky-Silver et al., 2003).

In some patients CoQ_{10} supplementation not only improved the clinical condition but also ameliorated the mitochondrial function. Indeed, respiratory chain activities returned to normal values in lymphocytes after 10 months of treatment in one previously reported patient (Rotig et al., 2000). In patients with myopathic features, lactic acid values normalized (Auré et al., 2004) and a normal CoQ_{10} content was observed in a second biopsy after 8 months of treatment (Di Giovanni et al., 2001). All these data suggest that exogenous ubiquinone restored the electron flow in the respiratory chain indicating its incorporation into mitochondria.

Whereas ubiquinone supplementation can dramatically improve the clinical condition of the patients, idebenone, a short chain quinone analogue, has little effect on ubiquinone biosynthesis defects. Indeed, the patient we previously reported was first given idebenone (5 mg/kg/d) and muscle weakness and ataxia initially improved (Rotig et al., 2000). After 2 months however, clinical symptoms worsened whereas plasma idebenone remained unchanged. The patient was then given ubiquinone and his condition continuously improved. Another patient reported by Auré et al., 2004 improved after 3 months of ubiquinone treatment. CoQ_{10} was then replaced by idebenone as neurological symptoms progressively appeared. However, during the four following months a clinical deterioration and increased lactatemia were observed. Reversion to the previous clinical status within three months was observed after return to initial CoQ_{10} treatment.

5. Genetic bases of ubiquinone deficiency

Little is known regarding CoQ_{10} biosynthesis in human but the biosynthesis pathway has been extensively studied

Table 5
Genes encoding proteins involved in ubiquinone synthesis

Gene			Prediction of mitochondrial targeting of human protein	
S. cerevisiae	Human	Chromosomal localization of human gene	Mitoprot	Maestro
COQ1	*PDSS1*	10p12.1	0.94	11.25
	PDSS2	6q21	0.84	7.50
COQ2	*COQ2*	4q21.23	0.99	19.86
COQ3	*COQ3*	6q16.3	0.98	16.15
COQ4	*COQ4*	9q34.13	0.99	13.86
COQ5	*COQ5*	12q24.31	0.97	15.46
COQ6	*COQ6*	14q24.3	0.94	13.93
COQ7	COQ7	16p12.3	0.78	27.83
COQ8	*COQ8*	1q42.13	0.99	21.37
COQ9	*COQ9*	16q12.2	0.99	23.17
COQ10	*COQ10A*	12q13.3	0.64	8.52
	COQ10B	2q33.1	0.98	8.52
	ADCK1	14q24.3	0.47	18.2951
	ADCK2	7q34	0.73	16.7574
	ADCK4	19q13.2	0.47	12.0477
	ADCK5	8q24.3	0.99	16.1012

The mitochondrial targeting is predicted by Mitoprot (http://ihg.gsf.de/ihg/mitoprot.html) or Maestro (Calvo et al., 2006).

in bacteria and in the yeast *S. cerevisiae* (Turunen et al., 2004). Briefly, the polyprenyl pyrophosphate chain deriving from mevalonate is condensed to the ring structure, 4-hydroxybenzoate. Based on protein homology, several genes have been identified in the human genome. All *S. cerevisiae* genes encoding proteins involved in ubiquinone synthesis and assembly (*COQ1-COQ10*) present human orthologs (Table 5). It should be mentioned that prenyl diphosphate synthase, the enzyme elongating the prenyl side chain of ubiquinone involved only one protein in the yeast *S. cerevisiae* encoded by *COQ1* gene (Ashby and Edwards, 1990), whereas in *Schizosaccharomyces pombe*, in mouse and in human, this enzyme involved two different subunits (Saiki et al., 2005). The human genes encoding these two subunits are *PDSS1* and *PDSS2*. Finally, *ADCK1-5* genes encode proteins homologous to aarF involved in ubiquinone synthesis in *Providencia stuartii* (Macinga et al., 1998). The exact function of most of these proteins is unknown in human; nevertheless they represent candidate genes for quinone deficiency in human. All the proteins encoded by these genes present mitochondrial targeting sequences.

Quinone deficiencies are most often suggested by deficient quinone-dependent respiratory chain activities and/or low amount of CoQ_{10} in the affected tissues. However, it should be hypothesized that none all these deficiencies are due to quinone biosynthesis pathway deficiency but could also be secondary to other yet unknown causes. Few reports attempted to identify the enzyme defect responsible for CoQ_{10} deficiency in patients. Incorporation of radiolabeled precursor of CoQ_{10} has been studied in the fibroblasts of only two cases. In a first report, we studied the incorporation of ^3H-mevalonate and observed a low level of decaprenol suggestive of prenyl diphosphate synthase deficiency, the enzyme elongating the prenyl side chain of CoQ_{10} (Rotig et al., 2000). Unfortunately, no mutation in the corresponding gene, *PDSS1*, could be identified. Recently, Artuch et al. reported a decreased incorporation of 4-[U-^{14}C] OH benzoate in the fibroblasts of a patient with cerebellar ataxia suggesting that the molecular defect might be allocated in the genes involved in the transprenylation pathway or in one of the steps following condensation of OH-benzoate and the prenyl side chain of ubiquinone (Artuch et al., 2006).

Finally, the first *COQ2* mutation was found in two patients with encephalomyopathy and nephropathy (Quinzii et al., 2006). This gene encodes the *para*-hydroxybenzoate-polyprenyltransferase that condensates the 4-OH benzoate ring with the prenyl side chain. Mutation in *PDSS2* gene, encoding the small subunit of prenyldisphosphate synthase has also been reported in a patient with Leigh syndrome and nephropathy (Lopez et al., 2006). Very recently, we also reported a single base pair *COQ2* deletion in a patient with fatal infantile multiorgan disease as well as a *PDSS1* missense mutation in two patients with a less severe multisystem disease (Mollet et al., 2007). Functional complementation of *Δcoq1* and *Δcoq2*-null

yeast strains demonstrated that both mutations are disease-causing.

6. Conclusion

Since the first report of ubiquinone deficiency in 1989, a large spectrum of phenotypes has been reported in this specific mitochondrial disorder. The clinical heterogeneity of ubiquinone deficiency is suggestive of a genetic heterogeneity that should be related to the large number of enzymes, and corresponding genes, involved in ubiquinone biosynthesis. Identification of disease-causing genes in patients will help elucidating the clinical variability of these rare conditions. Finally, whatever the underlying mutations, it is important that ubiquinone deficiency be recognized, because this form of mitochondrial dysfunction seems to respond well to oral CoQ_{10} administration.

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CoQ$_{10}$ deficiency diseases in adults

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Abstract

Deficiency of Coenzyme Q$_{10}$ (CoQ$_{10}$) in muscle has been associated with a spectrum of diseases including infantile-onset multi-systemic diseases, encephalomyopathies with recurrent myobinuria, cerebellar ataxia, and pure myopathy. CoQ$_{10}$ deficiency predominantly affects children, but patients have presented with adult-onset cerebellar ataxia or myopathy. Mutations in the CoQ10 biosynthetic genes, *COQ2* and *PDSS2*, have been identified in children with the infantile form of CoQ$_{10}$ deficiency; however, the molecular genetic bases of adult-onset CoQ$_{10}$ deficiency remains undefined.
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Keywords: Coenzyme Q; Deficiency; Mitochondria; CoQ; Adult

A lipid-soluble component of virtually all cell membranes, coenzyme Q$_{10}$ (CoQ$_{10}$) or ubiquinone is an isoprenylated benzoquinone. CoQ$_{10}$ transports electrons from complexes I and II to complex III in the mitochondrial respiratory chain and is essential for the stability of complex III (Santos-Ocana et al., 2002). It is also an antioxidant (Villalba and Navas, 2000) and is involved in multiple aspects of cellular metabolism (Turunen et al., 2004).

Primary CoQ$_{10}$ deficiency causes clinically heterogeneous diseases: (1) encephalomyopathy characterized by the triad of recurrent myoglobinuria, brain involvement and ragged-red fibers (Ogasahara et al., 1989; Sobreira et al., 1997; Boitier et al., 1998; Di Giovanni et al., 2001; Aure et al., 2004); (2) severe infantile multi-systemic disease (Rotig et al., 2000; Rahman et al., 2001; Salviati et al., 2005); (3) cerebellar ataxia (Musumeci et al., 2001; Lamperti et al., 2003; Gironi et al., 2004; Artuch et al., 2006); (4) Leigh syndrome with growth retardation, ataxia and deafness (Van Maldergem et al., 2002); and (5) isolated myopathy (Lalani et al., 2005; Horvath et al., 2006). These disorders are transmitted as autosomal recessive traits and in most cases respond to CoQ$_{10}$ supplementation. In most of the reported patients, the exact site and nature of the defects in the biosynthesis of CoQ$_{10}$ have not yet been identified. Because ubiquinone biosynthesis is complex and not fully defined, identification of the molecular genetic defect is not straightforward (Fig. 1).

The pathogenic molecular defect has been identified in only 3 patients with the infantile form of primary CoQ$_{10}$ deficiency: a homozygous mutation in the *COQ2* gene, which encodes 4-para-hydroxybenzoate:polyprenyl transferase, in two siblings with nephropathy and encephalopathy (Salviati et al., 2005; Quinzii et al., 2006), and compound heterozygous mutations in the *PDSS2* gene, which encodes subunit 2 of polyprenyl diphosphate synthase, the first enzyme of the CoQ$_{10}$ biosynthetic pathway (Fig. 1), in an infant with lactic acidosis, Leigh syndrome, and nephropathy (Lopez et al., 2006). The renal disease in all three cases manifested as steroid unresponsive nephrotic syndrome.

Among reported patients with presumed primary CoQ$_{10}$ deficiency, 12 are adults (Table 1) (Musumeci et al., 2001; Van Maldergem et al., 2002; Lamperti et al., 2003; Gironi et al., 2004; Horvath et al., 2006). In some adult patients, onset of the disease was during childhood, but Lamperti and colleagues described four with adult-onset ataxia

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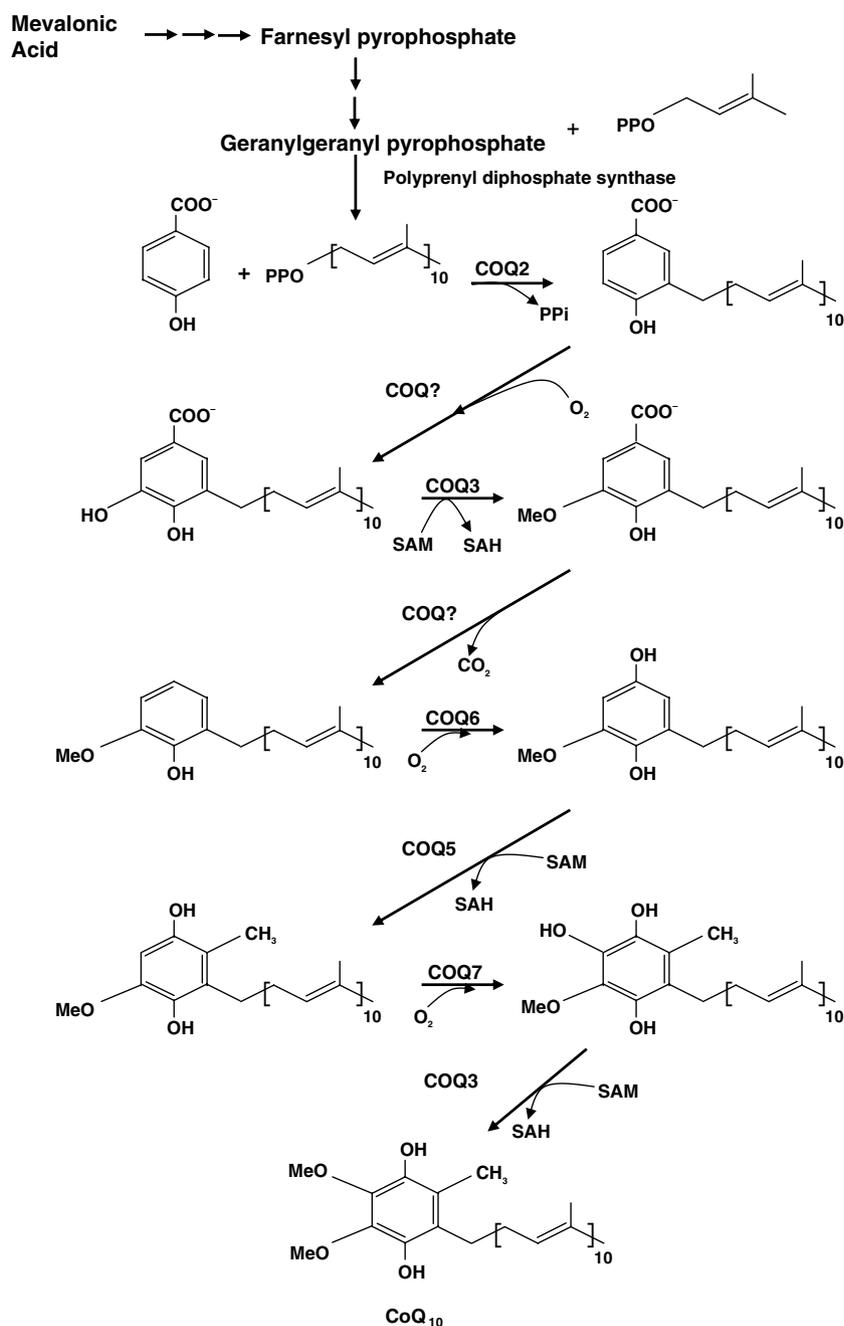
Fig. 1. CoQ$_{10}$ biosynthetic pathway with eight known biosynthetic enzymes denoted as polyprenyl diphosphosphate synthase (COQ1) and COQ2-8. CoQ$_{10}$ is composed of a benzoquinone and a decaprenyl side chain. While the quinone ring is derived from amino acids tyrosine or phenylalanine, the isoprenoid side chain is produced by addition of isopentenyl pyrophosphate molecules to geranylgeranyl pyrophosphate (derived from mevalonate pathway) by decaprenyl diphosphate synthase. After para-hydroxybenzoate and decaprenyl pyrophosphate are produced, at least seven enzymes (encoded by *COQ2-8*) catalyze condensation, methylation, decarboxylation, and hydroxylation reactions to synthesize CoQ$_{10}$.

among a cohort of eighteen patients with cerebellar ataxia and low CoQ$_{10}$ levels in muscle (Lamperti et al., 2003) while Gironi et al. and Horvath et al. reported four patients who presented at ages ranging from 29- to 39-years-old (Gironi et al., 2004; Horvath et al., 2006).

The adult patients with CoQ$_{10}$ deficiency reported by Musumeci et al. and Lamperti et al. had phenotypes similar to children with the ataxic form of CoQ$_{10}$ deficiency, namely, cerebellar ataxia and atrophy, associated with sei-

zures in 37% of the patients, pyramidal signs, mental retardation, weakness and motor development delay. Notably, the three adult patients reported by Lamperti and colleagues did not respond to CoQ$_{10}$ supplementation, whereas the three young adults described by Musumeci and colleagues showed dramatic improvements. All three affected siblings were wheelchair-bound, with alternating esotropia, severe limb ataxia with the slightest purposeful movement, peripheral neuropathies, and scoliosis. One

Table 1
CoQ$_{10}$ levels in muscle of 12 adults patients

	Age (years)	Patient muscle CoQ$_{10}$ levels	Control muscle CoQ$_{10}$ levels	Reference
Patient 1	20	7.4[a]	25 ± 3.5[a]	Musumeci et al. (2001)
Patient 2	25	6.6[a]		
Patient 3	24	7.1[a]		
Patient 4	24	7.1[a]		
Patient 5	31	43[b]	793[b]	Van Maldergem et al. (2002)
Patient 6	24	12.8[a]	27.6 ± 4.4[a]	Lamperti et al. (2003)
Patient 7	35	9.2[a]		
Patient 8	27	8.7[a]		
Patient 9	30	14.8[a]		
Patient 10	48	15.8[a]	27.6 ± 4.4[a]	Gironi et al. (2004)
Patient 11	35	13.5[a]		
Patient 11	33	0.6[c]	2.7–7[c]	Horvath et al. (2006)
Patient 12	29	0.8[c]		

[a] μg/gm fresh tissue (control values = means ± standard deviation).
[b] μg/gm protein (control value = mean).
[c] nmol/unit citrate synthase (control = range).

had generalized seizures and another had dystonia. After the proband began CoQ$_{10}$ supplementations at the age of 20 years, his strength and ataxia improved and he became able to walk a few steps. His siblings showed similar improvements. In addition, seizures in the affected sister disappeared on CoQ$_{10}$ therapy and her anti-convulsant medication was discontinued (Musumeci et al., 2001). In these three patients, we demonstrated that CoQ$_{10}$ deficiency was secondary to a stop codon mutation in the *APTX* gene, which is known to cause ataxia-oculomotor-apraxia 1 (AOA1) (Quinzii et al., 2005; Date et al., 2001; Moreira et al., 2001). Results from measuring CoQ$_{10}$ concentration in skeletal muscle from 12 additional patients from six different families with AOA1 confirmed this data (data not published). Intriguingly, both CoQ$_{10}$ and cholesterol share a common biosynthetic pathway, therefore, in AOA1, altered levels of these molecules could be due to aberrant biosynthesis. There is no obvious link between ataprataxin and regulation of CoQ$_{10}$ synthesis or catabolism. Nevertheless, we did not detect mutations in APTX genes in other 13 patients with cerebellar ataxia and CoQ$_{10}$ deficiency (Quinzii, DiMauro, Hirano, unpublished observation).

Van Maldergam and colleagues reported a 31-year-old woman and her older sister with the typical neuroradiological features of Leigh syndrome encephalopathy, growth retardation, infantilism, ataxia, deafness, and lactic acidosis, but unusually prolonged survival into adulthood. Both clinical and biochemical abnormalities improved remarkably with CoQ$_{10}$ supplementation (Van Maldergem et al., 2002).

Gironi and colleagues described two brothers with hypergonadotropic hypogonadism and progressive cerebellar ataxia, which started in the fourth decade of life. The late onset of ataxia and associated low levels of testosterone distinguish these patients from those previously reported by Musumeci et al. and Lamperti et al. (Gironi et al., 2004). As the synthesis of steroid hormones starts

with cholesterol, which has the same biosynthetic pathway as CoQ$_{10}$, it is conceivable that CoQ$_{10}$ and testosterone deficiencies may coexist; moreover, it is possible that hypergonadotrophic hypogonadism has been under-recognized as endocrine studies were not reported in described patients with the ataxic form of CoQ$_{10}$ deficiency. Both patients responded to CoQ$_{10}$ supplementation; they showed improved postural stability, gait and speech articulation, and testosterone returned to the normal range. The clinical improvements may have been related to increased muscle strength rather than amelioration of ataxia (Gironi et al., 2004). The lack of improvement of cerebellar functions with CoQ$_{10}$ supplementation may be due to initiation of therapy after irreversible structural changes have occurred in the brain. Alternatively, insufficient tissue distribution of CoQ$_{10}$, in particular its limited ability to cross the blood–brain barrier may explain the absence of therapeutic benefit in the CNS in these as well as in other patients subsequently reported (Aure et al., 2004; Lopez et al., 2006). Initial studies in rodents suggested that oral CoQ$_{10}$ supplementation increased levels in plasma, spleen, and liver, but not CNS (Reahal and Wrigglesworth, 1992; Zhang et al., 1996); however, subsequent publications demonstrated that long-term CoQ$_{10}$ administration increased brain mitochondrial concentrations, particularly in aged rodents (Matthews et al., 1998; Kwong et al., 2002).

The most dramatic improvement after CoQ$_{10}$ supplementation in adults has been associated with the pure myopathic form of CoQ$_{10}$ deficiency (Lalani et al., 2005; Horvath et al., 2006). The clinical presentation of this variant appears to be homogeneous, with subacute (3–6 months) onset of exercise intolerance and proximal weakness affecting predominantly the hip and shoulder girdle muscles. Serum CK and lactate levels were markedly increased. Histologic examination of skeletal muscle revealed a lipid storage myopathy with subtle signs of mitochondrial dysfunction. Biochemical measurement of the respiratory chain enzymes showed reduced activities of

complexes II and III (<50% of control mean) secondary to CoQ_{10} deficiency, as observed in all the variants of CoQ_{10} deficiency, and increased activity of citrate synthase, in keeping with mitochondrial proliferation. In the myopathic form, CoQ_{10} levels are low only in muscle, whereas in the infantile multi-systemic and in the cerebellar ataxic forms as well as in the patients described by Van Maldergam, CoQ_{10} is also reduced in fibroblasts.

CoQ_{10} deficiency can be also a secondary consequence of drugs, such as statins (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors). Statins have been used for the treatment of hypercholesterolemia and coronary artery disease and for the prevention of stroke. Their mechanism of action is the inhibition of cholesterol synthesis at the level of mevalonic acid. The biosynthetic inhibition is not selective, because statins impair the synthesis of other compounds that share mevalonate as precursor, such as dolichols and CoQ_{10}. For this reason, statin-related myopathy, manifesting as myalgia, muscle necrosis, and myoglobinura, has been hypothesized to be due to a partial deficiency of CoQ_{10} (Folkers et al., 1985; Rundek et al., 2004). Indirect support for this hypothesis comes from the first reported cases of CoQ_{10} deficiency, which presented as exercise intolerance, recurrent myoglobinuria, and encephalopathy (mental retardation and seizures) (Ogasahara et al., 1989; Sobreira et al., 1997; Boitier et al., 1998; Di Giovanni et al., 2001; Aure et al., 2004). Several groups have studied the effects of statins on the blood concentration of CoQ_{10} in patients with hypercholesterolemia and healthy subjects and there are several reports showing that various statins partially decrease CoQ_{10} levels in blood of patients with hypercholesterolemia and controls, although the number of subjects studied and the severity of CoQ_{10} deficiency varied markedly (Folkers et al., 1985; Rundek et al., 2004). Recently, Lamperti et al. address the question of whether levels of CoQ_{10} were also decreased in muscles of patients with statin-related myopathy (Lamperti et al., 2005). The authors measured CoQ_{10} concentration and respiratory chain enzyme activities in biopsied muscle from 18 patients with statin-related myopathy. Moreover, they looked for evidence of mitochondrial myopathy or morphologic evidence of apoptosis using the TUNEL assay. Their studies revealed a mild decrease in muscle CoQ_{10} concentration without histochemical or biochemical evidence of mitochondrial myopathy or morphologic evidence of apoptosis in most patients (Lamperti et al., 2005).

Finally, it noteworthy that reduced levels of CoQ_{10} in blood and mitochondria have been reported in Parkinson disease (PD) by a number of investigators (Shults and Haas, 2005). These data, together with the implication of oxidative damage and mitochondrial dysfunction in Parkinson disease and other neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS), Huntington disease (HD), and Friedreich's ataxia (FRDA) have stimulated interest in potential therapeutic effects of CoQ_{10} as an antioxidant (Beal, 2004). Initial small clinical trials have suggested beneficial effects in Parkinson disease and FRDA (Shults et al., 2002; Schapira, 2006). However, larger studies are necessary to better define the role of CoQ_{10} as primary or adjunctive therapy in neurodegenerative diseases.

The molecular bases and pathogenic mechanisms of the various primary and secondary forms of CoQ_{10} deficiency remain largely unknown. To date, primary CoQ_{10} deficiency has been genetically and biochemically proven just in few patients with infantile multi-systemic severe diseases, where nephropathy and encephalopathy seems to be the most consistent feature. However, CoQ_{10} deficiency should be considered in the differential diagnosis of subacute exercise intolerance and weakness and of all genetically undefined adult-onset cases of cerebellar ataxia, as well as in patients with AOA1, because CoQ_{10} supplementation seems to improve muscle weakness and other associated symptoms in some individuals. Further studies are likely to shed new insights into causes and to improve therapies for the multiple variants of CoQ_{10} deficiencies in adults and children.

Note Added in Proof

After the submission of this manuscript, three important papers on CoQ_{10} deficiency were published. Mollet et al. described new mutations, in PDSS1 and COQ2 as causes of primary deficiency in 3 patients with multisystemic infantile diseases (Mollet et al., 2007). Le Ber et al. confirmed the association between aprataxin mutations and low CoQ_{10} levels in muscle in 5 unrelated patients with ataxia and oculomotor apraxia 1 (Le Ber et al., 2007). Finally, mutations in the electron-tranferring-flavoprotein dehydrogenase (ETFDH) gene, were identified as a cause of the pure myopathic form of CoQ_{10} deficiency (Gempel et al., 2007).

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Review

Friedreich's ataxia: Coenzyme Q_{10} and vitamin E therapy

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Abstract

Since the identification of the genetic mutation causing Friedreich's ataxia (FRDA) our understanding of the mechanisms underlying disease pathogenesis have improved markedly. The genetic abnormality results in the deficiency of frataxin, a protein targeted to the mitochondrion. There is extensive evidence that mitochondrial respiratory chain dysfunction, oxidative damage and iron accumulation play significant roles in the disease mechanism. There remains considerable debate as to the normal function of frataxin, but it is likely to be involved in mitochondrial iron handling, antioxidant regulation, and/or iron sulphur centre regulation. Therapeutic avenues for patients with FRDA are beginning to be explored in particular targeting antioxidant protection, enhancement of mitochondrial oxidative phosphorylation, iron chelation and more recently increasing FRDA transcription. The use of quinone therapy has been the most extensively studied to date with clear benefits demonstrated using evaluations of both disease biomarkers and clinical symptoms, and this is the topic that will be covered in this review.
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Keywords: Friedreich's ataxia; Ataxia; Coenzyme Q_{10}; Mitochondria; Neurodegeneration

1. Clinical and pathological features

Friedriech's ataxia (FRDA) usually presents in adolescence and clinical features include a progressive limb and gait ataxia, absence of deep tendon reflexes, extensor plantar responses, loss of position and vibration sense in the lower limbs and dysarthria. Hypertrophic cardiomyopathy and skeletal abnormalities (including scoliosis and pes cavus) are relatively common, while diabetes and optic atrophy also have an increased incidence (Harding, 1981; Durr et al., 1996). Following the identification of the genetic mutation of the FRDA gene in 1996 (Campuzano et al., 1996), patients with late onset (Bidichandani et al., 2000), retained reflexes (Klockgether et al., 1996), spastic paraplegia (Gates et al., 1998), pure sensory ataxia (Berciano et al., 1997) and chorea (Hanna et al., 1998) have been shown to have the genetic abnormality.

To date measurement of clinical progression in FRDA has been limited and while there are several clinical ratings scales for patients with ataxia, none have been specifically designed for FRDA although the international co-operative ataxia ratings scale (ICARS) (Trouillas et al., 1997) has been most widely used and validated (Cano et al., 2005). Several studies have compared the relationship between the genetic mutation and clinical presentation (Durr et al., 1996; Filla et al., 1996), but there has been very little detailed analysis of the natural history of FRDA and how different factors influence disease progression.

Pathologically the most obvious findings are loss of large sensory neurones in the dorsal root ganglia and deterioration of the sensory posterior and Clarke's columns, spinocerebellar tracts and corticospinal motor tracts of the spinal cord (Harding, 1981). Cardiac hypertrophy is relatively common, and iron deposition in the heart has also been reported (Lamarche et al., 1993; Bradley et al., 2000).

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2. Genetic features

FRDA is inherited in an autosomal recessive pattern with over 95% of patients having a homozygous expansion of a GAA triplet repeat in intron 1 of the FRDA gene on chromosome 9 (Campuzano et al., 1996). Normal alleles usually have between 6 and 34 GAA repeats which can be expanded to between 70 and 1700 in patients or carriers (Durr et al., 1996). Some patients are compound heterozygotes with the GAA expansion in one allele and one of a variety of point mutations in the other allele.

The FRDA gene encodes a protein containing 210 amino acids. The pathological GAA repeat expansions in intron 1 result in decreased frataxin mRNA leading to decreased frataxin protein levels in patients (Bidichandani et al., 1998; Wong et al., 1999). This is thought to be caused by the GAA/TTC repeats which have been shown to form unusual DNA structures, including DNA triplexes, leading to blockade of transcription (see review by Patel and Isaya, 2001). The size of the GAA repeat appears to influence the clinical phenotype with a significant inverse relationship between the size of the smaller GAA repeat and the age of onset (Durr et al., 1996). In agreement with the lower levels of frataxin mRNA, frataxin protein levels were decreased in proportion to the GAA repeat size, with frataxin level correlating with the size of the smallest GAA expansion in FRDA lymphoblasts (Campuzano et al., 1997). This supports the inverse relationship between GAA size and age of onset.

Analysis of the frataxin protein sequence failed to identify any similarities with domains in other proteins with known function. Frataxin has a predicted N-terminal mitochondrial targeting sequence and has been shown to reside within the mitochondrion (Campuzano et al., 1997), however the normal function of frataxin remains elusive. X-ray crystallography and functional studies indicate that frataxin may bind both proteins and iron (Dhe-Paganon et al., 2000; Yoon and Cowan, 2003; Bulteau et al., 2004) suggesting it may have a role in mitochondrial iron metabolism.

3. Models of FRDA

Disruption or knock out of the yeast frataxin homologue (YFH1) gene in several yeast models resulted in mitochondrial iron accumulation, impaired mitochondrial respiratory chain function, decreased mitochondrial DNA levels and an increased susceptibility to oxidative stress induced by hydrogen peroxide (Babcock et al., 1997; Koutnikova et al., 1997; Foury and Cazzalini, 1997). This is consistent with a role of the yeast frataxin homologue protein (yfh1p) in mitochondrial iron homeostasis, antioxidant defence mechanisms or mtDNA regulation.

Frataxin has an important role in development as knockout of frataxin is lethal at an early stage of embryological development, making mouse models of FRDA more difficult to generate. Two conditional gene targeting models gave rise to mice lacking a full length FRDA transcript in heart and skeletal muscle (MCK mice) or decreased levels in the brain, liver and kidney and absent level in the heart (NSE mice) (Puccio et al., 2001). Clinically the NSE mice expressed a rapidly progressive movement disorder from approx. 12 days while the MCK mice exhibited weight loss at 7 weeks followed by progressive signs of muscle fatigue. These models exhibited signs of cardiac hypertrophy, mitochondrial respiratory chain and aconitase dysfunction and iron accumulation (Puccio et al., 2001) but lacked evidence of oxidative damage (Seznec et al., 2005). This contrasted with a human frataxin YAC transgenic mouse model demonstrating neurodegeneration and cardiac iron deposition with increasing age, where oxidative damage was the main biochemical change (Al-Mahdawi et al., 2006).

4. Mitochondria, iron and oxidative stress in FRDA

Frataxin has clearly been shown to reside in the mitochondria, although there is now evidence of a small cytosolic pool of protein (Condo et al., 2006). In keeping with its mitochondrial location, deficiency of frataxin protein is associated with a decrease in mitochondrial respiratory chain complexes I, II and III and aconitase activities in post mortem heart and skeletal muscle from FRDA patients (Bradley et al., 2000).

^{31}Phosphorous magnetic resonance spectroscopy (^{31}P MRS) is an in vivo technique that can, amongst other parameters, measure the high energy phosphorous compounds phosphocreatine (PCr) and ATP in heart and skeletal muscle. In skeletal muscle in particular, the analysis of the rate at which PCr recovers following exercise (V_{max}) is a measure of the efficiency of oxidative phosphorylation (Kemp et al., 1993) and in heart the PCr/ATP ratio is a good measure of energy availability (Ingwall et al., 1985). ^{31}P MRS analysis of FRDA patients has revealed markedly decreased oxidative phosphorylation in the heart (Lodi et al., 2001) and skeletal muscle, with the latter correlating with the size of the GAA1 repeat (Fig. 1, Lodi et al., 1999). These data underline the role of mitochondrial dysfunction in FRDA and suggest it is playing a primary role in disease pathogenesis.

The evidence of oxidative stress and damage in FRDA is quite extensive. It was first implicated when deficiency of the antioxidant vitamin E, caused by mutations of the alpha tocopherol transfer protein gene, was shown to cause a similar phenotype which responded to vitamin E therapy (AVED, Cavalier et al., 1998). As mitochondria have a very high vitamin E content (Buttriss and Diplock, 1988), and deficiency leads to impaired respiratory chain function (Thomas et al., 1993), it has been suggested that increased oxidative damage to the mitochondria may be the common mechanism between AVED and FRDA.

Various markers have indicated increased oxidative stress and damage in FRDA patients including; raised urine levels of 8-hydroxy-2′-deoxyguanosine (8OH2′dG)
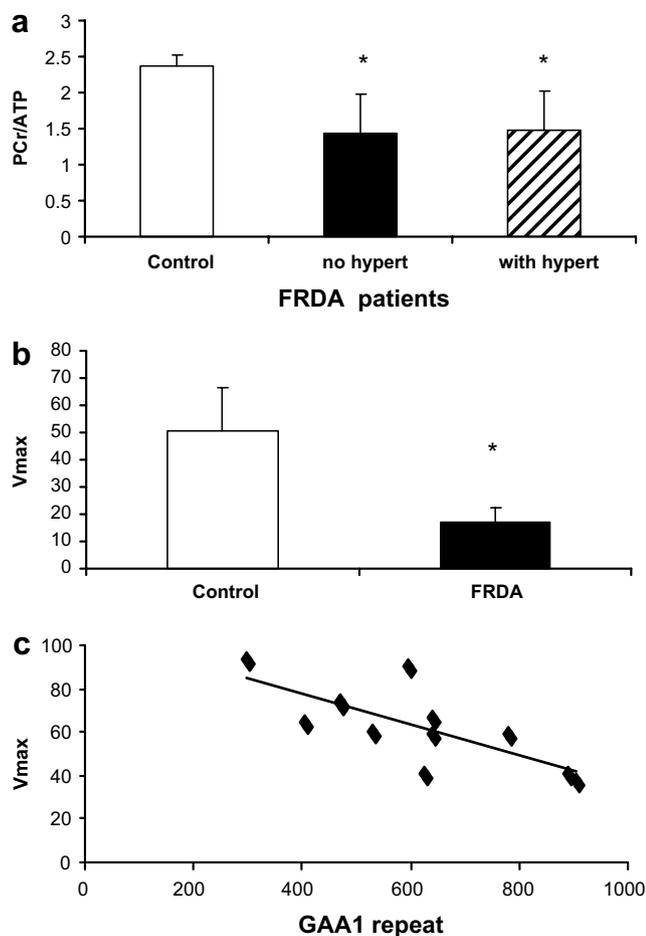
Fig. 1. ^{31}P MRS data of cardiac and skeletal muscle in patients with Friedreich's ataxia and its relationship to genetic severity. (a) Cardiac PCr/ATP ratios, controls ($n = 18$), FRDA patients were divided into those with hypertrophy ($n = 8$) and those without ($n = 10$) (b) skeletal muscle V_{max} recover following exercise ($n = 12$) (c) relationship between skeletal muscle V_{max} recovery rates and GAA1 repeat size, linear regression, $r = -0.75$, $p = 0.004$. Unpaired t test *$p < 0.0001$. Modified from Lodi et al. (1999, 2001).

suggesting elevated oxidative damage to DNA; decreased free glutathione levels in blood suggesting extensive gluathionylation of proteins in response to oxidative stress (Piemonte et al., 2001) and raised plasma malondialdehyde (MDA) levels indicative of increased lipid peroxidation (Emond et al., 2000; Bradley et al., 2004). The analysis of fibroblasts from FRDA patients suggests frataxin deficiency leads to a delayed antioxidant defence response, (Jiralerspong et al., 2001) which may relate to the increased sensitivity to free radical generation in yeast YFH1 knockout models and cultured fibroblasts from FRDA patients (Babcock et al., 1997; Bradley et al., 2004).

In agreement with the accumulation of mitochondrial iron in yeast YFH1 knockout models iron deposits have been detected in heart and liver from some but not all FRDA patients (Bradley et al., 2000; Lamarche et al., 1993). Consequently, rather than reflecting the antioxidant capacity of the cells it is possible that the increased sensitiv-ity to oxidative stress in FRDA models reflects an increased mitochondrial labile iron content promoting Fenton chemistry and ensuing cell damage.

5. Disease mechanisms

The presence of increased mitochondrial iron, decreased respiratory chain activity and oxidative damage are common features in yeast and mouse models of frataxin deficiency and samples from FRDA patients. The primary role of frataxin and the primary consequences of its deficiency remain unresolved, however, there are important clues that are helping to delineate the chronology of these events.

The pattern of respiratory chain dysfunction in FRDA is reminiscent of that observed in situations where oxidative stress has been implicated including a manganese superoxide dismutase knockout transgenic mouse model (Melov et al., 1999), and Huntington's disease (Tabrizi et al., 2000). Consistent with the idea that frataxin deficiency leads to increased mitochondrial iron levels and subsequent oxidative damage and mitochondrial respiratory chain dysfunction, chelating iron in the medium of yeast cells lacking yfh1p prevented mitochondrial iron accumulation and improved respiratory chain activities (Foury, 1999). This suggested that the mitochondrial iron accumulation was responsible for the decrease in respiratory chain function but the continued decrease in aconitase activity implied an alternative cause of the aconitase inhibition. This primary role of oxidative damage in FRDA contrasts with the lack of oxidative stress and damage markers in the MCK and NSE conditional mouse models (Seznec et al., 2005). This may reflect the focal loss of frataxin where the oxidative damage may be more restricted and less easily detected, or alternatively suggests the oxidative damage is a secondary response and requires the presence of residual frataxin levels. The presence of oxidative damage as the main biochemical consequence of decreased frataxin levels in another mouse model (Al-Mahdawi et al., 2006) also raises doubt about the significance of this feature of the conditional models.

Mitochondria play a pivotal role in cellular iron handling. With iron sulphur (Fe–S) centre (Lill et al., 1999) and haem biosynthesis pathways located within the mitochondrion, iron uptake needs to be tightly regulated in line with these processes. The increase in mitochondrial iron in the various models of FRDA and in patient samples is consistent with a role of frataxin in iron handling. The pattern of deficiency in FRDA involving Fe–S containing activities (complexes I–III and aconitase), while sparing cytochrome oxidase activity (which contains haem), was consistent with an abnormality of Fe–S centre but not haem synthesis. The proposed role of frataxin in Fe–S centre synthesis (Duby et al., 2002) is consistent with the secondary accumulation of mitochondrial iron which has also been reported in yeast mutants with defective iron sulphur synthesis (Kispal et al., 1997; Lange et al., 2000; Li et al., 1999; Knight et al., 1998).

In addition to a possible interaction with components of the Fe–S synthesis machinery (Yoon and Cowan, 2003; Stehling et al., 2004) more specific roles for frataxin have been suggested following interactions between frataxin and succinate dehydrogenase (Gonzalez-Cabo et al., 2005), or aconitase (Bulteau et al., 2004) where it may have a regulatory role.

While the exact pathological mechanisms of FRDA are not understood there are a number of ways the biochemical abnormalities observed in FRDA can interact. Mitochondrial iron levels may increase due to decreased Fe–S synthesis leading to increased sensitivity to oxidative stress. Aconitase is an iron sulphur protein whose activity is particularly sensitive to free radical damage (Hausladen and Fridovich, 1994), consequently the decreased aconitase activities reported in heart and skeletal muscle from FRDA patients (Bradley et al., 2000; Rotig et al., 1997) may reflect elevated free radical damage and/or decreased Fe–S centre synthesis or repair. Likewise if Fe–S centre synthesis is impaired the resulting decrease in respiratory chain function could lead to elevated free radical generation and oxidative stress (Hasegawa et al., 1990).

While the exact role frataxin plays in the mitochondrion remains elusive, mitochondrial respiratory chain dysfunction, oxidative stress and iron accumulation appear to be exacerbated with disease progression, and are therefore useful targets for therapeutic intervention.

6. Coenzyme Q_{10} and vitamin E in ataxia

Coenzyme Q_{10} (CoQ_{10}) and vitamin E are important mitochondrial antioxidants and deficiencies of both have been implicated in ataxia. Decreased muscle CoQ_{10} levels were reported in patients with genetically undefined cerebellar ataxia, (Lamperti et al., 2003) suggesting CoQ_{10} depletion may be a secondary phenomenon in diseases involving ataxia. In another study ICARS scores for patients with ataxia associated with a severe muscle CoQ_{10} deficiency improved after 1 year of oral CoQ_{10} therapy suggesting it played a significant role in pathogenesis and was responsive to therapy (Musumeci et al., 2001). Vitamin E is obtained solely from the diet and vitamin E deficiency caused by mutation of the alpha tocopherol transferring protein leads to ataxia (Ouahchi et al., 1995) which is responsive to vitamin E therapy (Kayden, 1993).

7. Therapeutic intervention

When approaching the issue of therapeutic intervention in a slowly progressive neurodegenerative disorder like FRDA there are many issues to consider including; type of therapy, whether any clinical effect will be restricted to modifying disease progression, the natural rate of clinical deterioration and therefore the duration of therapy required to show any benefit. The choice of therapy has predominantly been based upon what is understood about the disease mechanisms and the ability of the therapy to gain access to neurological tissues. Consequently therapeutic intervention has focussed on iron chelation, antioxidant protection and mitochondrial energy enhancement.

FRDA is associated with both neurological and non-neurological symptoms. The former are associated with neuronal loss and so therapy is only likely to slow their progression, other symptoms however, may demonstrate improvement with therapeutic intervention. The paucity of data relating to the validation of rating scales for the assessment of FRDA patients and also a lack of validated natural history data to predict disease progression has led to variations in trial design. Published trials have usually been open label trials with durations ranging between 6 months and 4 years and assessing clinical progression using ICARS, echocardiography and occasionally using ^{31}P MRS as a biomarker of mitochondrial bioenergetics.

8. Antioxidant therapy

8.1. Idebenone

Idebenone is an analogue of coenzyme Q_{10} with a short isoprene side chain, it is well tolerated by humans, crosses the blood brain barrier (Nagai et al., 1989), has been reported to be a relatively good antioxidant (Mordente et al., 1998), and has been used in a variety of diseases with some benefits (Gutzmann and Hadler, 1998; Ranen et al., 1996). Idebenone therapy decreased the cardiac hypertrophy (as determined by echocardiography) in the majority of patients (Hausse et al., 2002) but there was no influence upon clinical progression (ICARS) (Hausse et al., 2002; Mariotti et al., 2003). Another assessment of idebenone failed to identify improvements in skeletal muscle bioenergetics (^{31}P MRS) or echocardiographic parameters (Schols et al., 2001), although this may reflect the short time scale used.

9. Prolonged vitamin E and CoQ_{10} therapy

Vitamin E is a naturally occurring lipid soluble antioxidant distributed throughout cellular membranes but predominantly in mitochondrial membranes. It is obtained in the diet and vegetable oils and nuts provide a particularly rich source. Vitamin E treatment has been shown to increase vitamin E levels in a variety of tissues including brain, muscle and heart (Zhang et al., 1995). It has been used to treat cardiovascular disease, Parkinson's disease, cancers and AVED with varying degrees of success (Stephens et al., 1996; Shoulson, 1998; Bostick et al., 1993; Gabsi et al., 2001), however, in FRDA its efficacy has only been assessed in conjunction with coenzyme Q_{10}.

As mitochondrial respiratory chain dysfunction is a common feature in FRDA patients and various model systems, drugs which enhance mitochondrial ATP synthesis are good candidates for therapeutic intervention. As an electron carrier in the respiratory chain CoQ_{10} has been used to enhance cellular energy status. While CoQ_{10} is
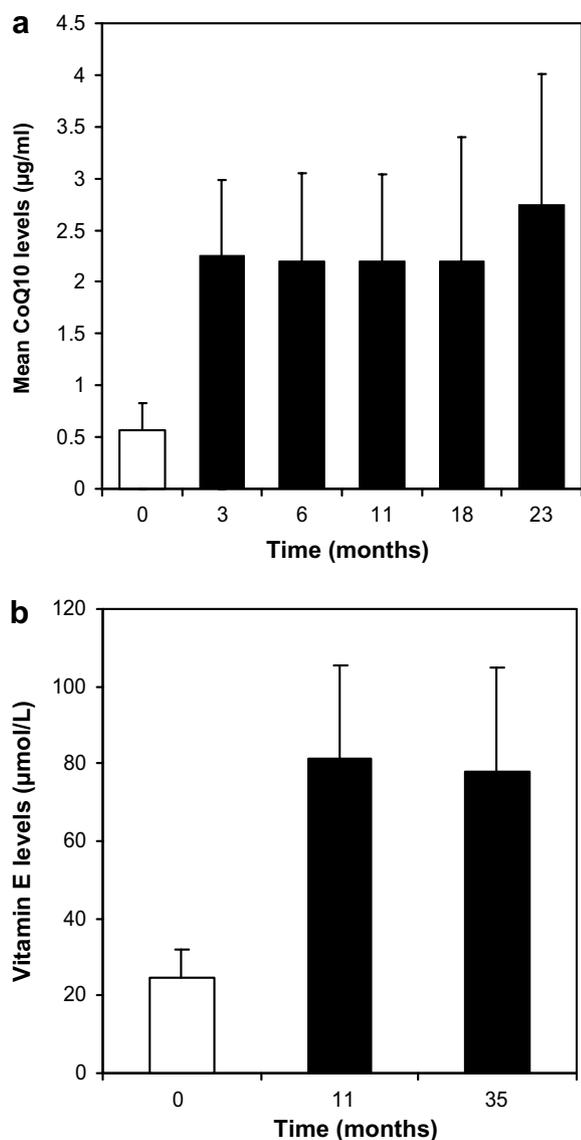
Fig. 2. Influence of therapy upon serum levels of (a) vitamin E and (b) CoQ_{10} during the trial. Values represent mean $\pm$ SD, $n = 10$, open bars represent the pre-therapy levels.

obtained in the diet it is also synthesised in all cells via a pathway that shares some of the steps involved in cholesterol biosynthesis. CoQ_{10} is readily taken up into the blood (Folkers et al., 1994), the brain (Matthews et al., 1998) and liver (Zhang et al., 1995) although other reports suggest dietary CoQ_{10} levels do not influence tissue CoQ_{10} levels in the rat (Reahal and Wrigglesworth, 1992). It is interesting to note that in rats fed two different doses of CoQ_{10} for varying lengths of time it was the duration of the diet rather than the dose of CoQ_{10} that was the most important variable when predicting the increase in tissue CoQ_{10} levels (Sohal et al., 2006).

CoQ_{10} has been beneficial in several neurological diseases including Parkinson's disease (Schulz et al., 2000) and ataxia associated with markedly decreased CoQ_{10} levels (Musumeci et al., 2001). CoQ_{10} is believed to be involved in the reduction of oxidised vitamin E and therefore when combined in a therapy they may act synergistically (Ernster and Dallner, 1995). This was found to be the case in protecting rats against atherosclerosis (Thomas et al., 2001).

The combination of CoQ_{10} and vitamin E has been shown to be well tolerated and therefore is ideal for a long term study of a high dose therapy for FRDA. A 4 year open label trial has been completed with 10 genetically confirmed FRDA patients given 2100 IU/day vitamin E and 400 mg/day CoQ_{10} (Hart et al., 2005) and the data will be reviewed here. The International Cooperative Ataxia Ratings Scale was used to evaluate clinical progression, in addition to biomarkers of in vivo mitochondrial bioenergetics (cardiac and skeletal muscle ^{31}P MRS) and cardiac hypertrophy (echocardiography) to evaluate the effect of therapy. During the course of the therapy all patients demonstrated an increase in serum vitamin E (2.2–6-fold increase over baseline) and CoQ_{10} levels (2.3–7.4-fold increase over baseline), (Fig. 2) demonstrating good bioavailability. Most remarkable was the improvement in cardiac (phosphocreatine:ATP ratio) and skeletal muscle (post exercise maximum rate of mitochondrial ATP synthesis, V_{max}) bioenergetics after 3 months of therapy which was maintained throughout the 4 years of the trial (Hart et al., 2005). This clearly demonstrated that the combined vitamin E and CoQ_{10} therapy had a significant and prolonged benefit upon the defective mitochondrial function in these peripheral tissues. While the prolonged improvement in cardiac bioenergetics did not have any impact upon cardiac hypertrophy, fractional shortening (FS) showed a progressive improvement that reached significance after 3 years (Hart et al., 2005).

In the absence of a direct evaluation of mitochondrial function in neurological tissues changes to the patients' ICARS scores were the only markers of neurological function. The clinical scores did not increase over the 4 years of the trial suggesting the disease progression was stabilised (Hart et al., 2005). A closer analysis of the component scores of the ICARS revealed that the posture and gait subscores increased significantly from the pre-therapy scores indicating clinical progression of these symptoms, while the kinetic subscores decreased ((Hart et al., 2005), although not statistically significant), indicating clinical improvement.

Interpretation of the clinical data from a trial such as this one is complicated because patients with FRDA are a heterogeneous group with variables including age, disease onset, disease duration, GAA repeat size, clinical presentation and severity. This is not only reflected in the variable presentation at the beginning of the trial but will also influence their disease progression. When combined with a lack of comprehensive natural history data interpretation of a small trial like this one is quite difficult. To address this disease progression was predicted using cross-sectional data from 77 FRDA patients divided into four groups according to their GAA1 repeat size to account for the influence of

genetic severity on disease progression. The rate of clinical change for each patient over the 4 years of the trial was determined and compared with those predicted for the appropriate cross-sectional group. The change in clinical symptoms (total ICARS scores) were better than predicted in six patients and indeed demonstrated an improvement (Fig. 3). When the different components of this score were analysed it was clear that the kinetic scores paralleled those for the total ICARS scores, with the same six patients demonstrating an improvement and an additional patient with a more stable clinical picture than predicted, but the change in posture and gait scores were similar to the predicted scores for all except one patient who showed an improvement implying not all symptoms responded to this therapy (Hart et al., 2005).

Decreased energy supply appears to be an important early event in FRDA (Lodi et al., 1999). The improved car-

Fig. 3. Rates of change of total ICARS scores plotted against patient age during the 4 years of therapy. Dotted lines represent the mean rate of change calculated from cross-sectional data for patients with (a) >900 GAA1, $n = 22$ (b) 601–750 GAA1, $n = 18$ (c) <600 GAA1, $n = 20$. The solid lines represent the rate of change for individual patients plotted from the data taken at 0, 6, 12, 18, 24, 36 and 48 months during the therapy. Asterisk represents patients with rates of change in ICARS scores better than predicted (outside 95% CI) from the cross-sectional data.

diac and skeletal muscle MRS data clearly demonstrated that the therapy caused a sustained improvement in heart bioenergetics. While this was associated with improved fraction shortening there was no impact upon the degree of hypertrophy evident before therapy was started. However, little is documented about the natural history of the cardiac hypertrophy in FRDA and therefore it was not possible to determine if the therapy prevented progression of the hypertrophy. While there is some debate as to the effect of increased dietary CoQ_{10} and vitamin E upon tissue levels of these chemicals (Bhagavan and Chopra, 2006; Ibrahim et al., 2000) the improved heart and skeletal muscle ^{31}P MRS features implies they are at least effectively targeting these tissues and had an influence upon clinical progression in at least a proportion of patients.

The lack of apparent benefit of CoQ_{10}/vitamin E therapy on cardiac hypertrophy contrasted with that reported for idebenone therapy (Hausse et al., 2002; Mariotti et al., 2003; Buyse et al., 2003). This may relate to differences in patient selection, in particular the degree of cardiac hypertrophy prior to therapy was relatively mild in the vitamin E/CoQ_{10} study, alternatively the tissue penetrance of the agents may differ. The differences did not relate to bioenergetic improvement as in contrast to this therapy, idebenone did not enhance oxidative phosphorylation (Schols et al., 2001). Conversely, the apparent clinical benefits of the CoQ_{10}/vitamin E therapy, which have not been reported for idebenone therapy, may be related to the improvements in bioenergetics.

10. Conclusion

In conclusion, there is still some debate as to the exact pathological mechanisms involved in FRDA. However, the involvement of defective mitochondrial energetics and increased oxidative damage is widely accepted. The use of a high dose quinone therapy, either Idebenone or CoQ_{10} plus vitamin E have been shown to have clinical benefits. In particular Idebenone has decreased the cardiac hypertrophy and CoQ_{10} plus vitamin E caused a prolonged improvement in cardiac and skeletal muscle bioenergetics and clinical scores were improved in 7 out of 10 patients. Larger randomised controlled trials with improved longitudinal data are required to confirm the findings of these trials and to help interpret the benefits of these therapies in a wider population of patients.

Acknowledgements

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The evidence basis for coenzyme Q therapy in oxidative phosphorylation disease

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Abstract

The evidence supporting a treatment benefit for coenzyme Q_{10} (CoQ_{10}) in primary mitochondrial disease (mitochondrial disease) whilst positive is limited. Mitochondrial disease in this context is defined as genetic disease causing an impairment in mitochondrial oxidative phosphorylation (OXPHOS). There are no treatment trials achieving the highest Level I evidence designation. Reasons for this include the relative rarity of mitochondrial disease, the heterogeneity of mitochondrial disease, the natural cofactor status and easy 'over the counter availability' of CoQ_{10} all of which make funding for the necessary large blinded clinical trials unlikely. At this time the best evidence for efficacy comes from controlled trials in common cardiovascular and neurodegenerative diseases with mitochondrial and OXPHOS dysfunction the etiology of which is most likely multifactorial with environmental factors playing on a background of genetic predisposition. There remain questions about dosing, bioavailability, tissue penetration and intracellular distribution of orally administered CoQ_{10}, a compound which is endogenously produced within the mitochondria of all cells. In some mitochondrial diseases and other commoner disorders such as cardiac disease and Parkinson's disease low mitochondrial or tissue levels of CoQ_{10} have been demonstrated providing an obvious rationale for supplementation. This paper discusses the current state of the evidence supporting the use of CoQ_{10} in mitochondrial disease.
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1. Introduction

The concept of evidence-based medicine was first introduced to the medical community in 1992 by a working group of the American Medical Association (Evidence Based Medicine Working Group, 1992). Evidence-based medicine is the practice of medicine based upon the best available scientific data which means basing decisions on the most accurate and reproducible medical facts (Brown Melissa et al., 2005). The idea that treatments for disease should only be advocated when there is good evidence supporting their safety and efficacy is a basic tenet of modern medicine. However, high-quality evidence is most readily obtained in common socioeconomically important diseases of interest to society and the pharmaceutical industry. Comparatively rare genetic diseases are much harder to study for practical and fiscal reasons.

The authors of the original concept of evidence-based medicine pointed out that 'evidence-based medicine does not advocate a rejection of all innovations in the absence of definitive evidence. When definitive evidence is not available, one must fall back on weaker evidence' (Evidence Based Medicine Working Group, 1992).

The best available scientific data in rare disorders are often of a much lower quality than is desirable however clinicians must nevertheless make treatment decisions based upon these data. When making treatment decisions it is important also to consider the concept of value-based medicine which moves beyond the evidence base to take in to

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account improvement in quality of life and/or length of life of a health care intervention (Brown Melissa et al., 2005). In the case of coenzyme Q_{10} (CoQ_{10}) treatment, the concept of value-based medicine has particular meaning as the cost of this supplement is high, with current data on dosage levels and efficacy many insurance companies will not pay for the treatment and benefit to the patient if present may be more prevention of deterioration rather than a perceived improvement in quality of life. This article will discuss the current quality of the evidence supporting the use of CoQ_{10} in primary mitochondrial disease (mitochondrial disease) which may be defined as a heterogeneous group of disorders characterized by impaired energy production due to genetically based oxidative phosphorylation dysfunction (OXPHOS).

2. Levels of evidence and important design factors in treatment trials

The international guidelines for grading the level of evidence in interventional studies were first proposed by Sackett (1993).

Level I evidence is considered the most reliable type of data. Achieving this evidence level requires a randomized clinical trial with a type 1 error [α] < or equal to 0.05 and type 2 error [β] < or equal to 0.2. An α of 0.05 is a 1/20 probability of a false positive result. A low β ensures that the study is adequate powered.

Meta-analysis is an increasingly utilized research method for combining data from a number of level II clinical trials in order to increase power and generate level I evidence.

Level II evidence also requires a randomized clinical trial but the α is high >0.05 and/or the β is high >0.20. Level II studies may show a non-statistically significant trend towards patient improvement but are under powered.

Level III evidence comes from studies which are uncontrolled non-randomized clinical trials with the treatment group often compared to a historical control group.

Level IV evidence is obtained from case series without a control group.

Level V evidence results from case reports.

Important trial design factors should also be taken into account in the interpretation of the value of a clinical trial. These include the dropout rate, an intention to treat approach, the degree of masking or blinding in the trial, comparability of the study and control population and the source of funding. The ideal drug treatment clinical trial is an adequately powered randomized double-blind placebo-controlled trial.

3. Problems in achieving Level I evidence in mitochondrial disease

Examining the requirements for Level I evidence of a therapeutic intervention in mitochondrial disease we encounter a number of significant problems.

These are as follows:

(1) Although mitochondrial diseases are thought to have a minimum live birth incidence of 1 in 5000 (Skladal et al., 2003; Sanderson et al., 2006) these are still rare disorders compared to common diseases such as diabetes, stroke, coronary artery disease and the neurodegenerative disorders of aging. Even for these common diseases treatment studies require expensive multi-center trials in order to be adequately powered. Only for treatments which are dramatically effective will small-scale trials prove adequate. No such treatments exist for mitochondrial disease.

(2) The problem of the small number of patients available for study is compounded by the heterogeneity of mitochondrial disease. With mitochondrial DNA (mtDNA) diseases heteroplasmy further complicates the situation as organ specific mutational load defines the phenotype. Thus matching the control and study populations is very difficult. To some extent use of a crossover trial design can help alleviate inter-patient phenotypic variability.

(3) Defining outcome measures in treatment trials of mitochondrial disease is difficult. Particularly when they affect the brain, mitochondrial disorders are characterized by episodic deterioration often precipitated by stress such as intercurrent viral illness. Quantitation of numbers of hospitalizations or episodes of deterioration requires a long time course of study and large numbers of patients. Improvement in biochemical parameters such as plasma lactate levels has not been shown to correlate with clinical outcome or symptomatic improvement (Barshop et al., 2004; Vissing et al., 2001). In a recent blinded placebo-controlled crossover trial of dichloroacetate in MELAS A3243G mutation patients, which was prematurely discontinued because of neuropathy, a composite global clinical outcome scale was used as a primary outcome measure. This Global Assessment of Treatment Efficacy (GATE) score was a 5-point scale determined by clinical investigator consensus at the end of each 6-month follow-up period based on four components; a neurological examination semi-quantitatively rated with the Columbia Neurologic Score, a neuropsychological performance score, a health-related event inventory and the Karnofsky score of daily living functional abilities (Kaufmann et al., 2006). Use of this composite score highlights the difficulty of assessing outcome even in one of the most phenotypically uniform mtDNA diseases.

(4) The relative rarity of primary mitochondrial disease places these patients below the radar of the pharmaceutical industry and so large scale funding of clinical trials of promising new agents is not likely in the near future and has not occurred in the past. CoQ_{10} is not patentable as a natural cofactor and thus major funding for clinical trials is only likely to come from the FDA, the NIH or other government granting agency.

(5) There are occasions when the performance of a placebo-controlled clinical trial is inappropriate. This is the case with primary CoQ_{10} deficiency diseases where case ser-

ies (Level IV evidence) and case reports (Level V evidence) clearly demonstrate that treatment with CoQ_{10} significantly alters the course of the disease and can be lifesaving (Boitier et al., 1998; Ogasahara et al., 1989; Rotig et al., 2000; Salviati et al., 2005; Lalani et al., 2005; Musumeci et al., 2001; Artuch et al., 2006; Di Giovanni et al., 2001). In these rare disorders of primary deficiency a placebo-controlled clinical trial cannot and should not be performed. In mitochondrial disease not due to primary CoQ_{10} deficiency however the benefit of CoQ_{10} treatment has not been convincingly established and Level I clinical trials are needed.

4. Why might CoQ_{10} be helpful in mitochondrial disease?

Throughout this supplement of 'Mitochondrion' the multiple cellular functions of CoQ_{10} have been discussed. Synthesized in mitochondria CoQ_{10} is transported to membranes throughout the cell where it participates as a redox molecule. It is involved as an antioxidant and in signaling at the plasma membrane. Quinones modulate the mitochondrial permeability transition pore regulating apoptosis. As an antioxidant CoQ_{10} regenerates α-tocopherol (Lass et al., 1999) and ascorbate. Its pro-oxidant role is important in cell signaling and in mitochondria and in cell membranes it functions as an electron transport molecule. All of these Q functions are important for cell homeostasis and survival. Several of these multiple functions of CoQ_{10} could prove beneficial if tissue levels are enhanced by supplementation. There is evidence that aging (Rebrin et al., 2004; Corral-Debrinski et al., 1992a,b) and primary mitochondrial disease with OXPHOS impairment are associated with oxidative injury to protein, DNA and lipids (Wallace et al., 1995; Mecocci et al., 1993; Corral-Debrinski et al., 1992a,b; McKenzie et al., 2004; Wei et al., 2001). With impaired ATP production apoptosis can be triggered and this will result in cell loss both in normal aging and in mitochondrial disease. Low levels of mitochondrial CoQ_{10} in disease states can be expected to further impair OXPHOS function. For orally administered treatment two basic questions require answers. Can exogenously administered CoQ_{10} raise tissue levels and is there evidence of CoQ_{10} deficiency in mitochondrial disease?

5. Tissue penetration of exogenously administered CoQ_{10}

5.1. Animal studies

In guinea pigs given a single IV dose of $[^{14}C]CoQ_{10}$ (the natural quinone in this species) isotope uptake in brain, heart, adrenal and kidney was demonstrated (Yuzuriha et al., 1983). A typical human dose in mitochondrial disease is of the order of 5–15 mg/kg/d however direct comparisons of animal dosing cannot be made with human doses without correction for allometric scaling (see www.fda.gov/cder/cancer/animalframe.htm). A human dose of 10 mg/kg equates to a mouse dose of 30 mg/kg, a

rat dose of 60 mg/kg and a guinea pig dose of 77 mg/kg. Studies in rats (Scalori et al., 1990) and mice (Lass et al., 1999) suggested that enterally administered CoQ_{10} increased levels in blood and liver but not in other tissues. Zhang et al. studied rats using a dose of 10.4 mg/kg/d. and found uptake of only 2–3% of the administered dose with elevation in plasma and liver but not heart or kidney confirming the earlier studies of rodent tissue uptake (Zhang et al., 1995). Isotopic uptake studies of CoQ_{10} administered intraperitoneally in rats found little uptake in kidney, muscle and brain (Bentinger et al., 2003) however higher dose studies of CoQ_{10} administered enterally in rats (150 mg/kg/d) have shown liver, kidney, heart and brain tissue and mitochondrial uptake (Kwong et al., 2002). Using much higher doses of up to 654 mg/kg/d in young mice Kamzalov et al. demonstrated a doubling of tissue levels of total CoQ_{10} in skeletal muscle and brain with a 3-fold increase in liver and 61% increase in heart. Much smaller increases were found in mitochondria from these tissues with the greatest increase in heart and skeletal muscle mitochondria. α-Tocopherol tissue levels were increased in all tissues studied by CoQ_{10} treatment with skeletal muscle homogenates showing a 60% increase and liver a 100% increase (Kamzalov et al., 2003). Mitochondrial levels of CoQ are up to 5-fold higher than tissue homogenates and mitochondrial measurements of CoQ content appear the most reliable indicator of tissue uptake (Rebrin et al., 2004). Thus it appears that rats and mice differ from each other and from other species in tissue uptake of exogenously administered CoQ_{10} however findings in rodents are not readily transferable to humans as CoQ_9 is the major quinone in these species.

5.2. What is the human data supporting tissue uptake of enterally administered CoQ_{10}?

It is clear that bioavailability, dosage and duration of treatment are important factors.

There are few data on tissue CoQ_{10} levels after treatment in humans however there are some cardiac and muscle direct measurements of post-treatment tissue levels. Patients were randomized to placebo or 300 mg/d of CoQ_{10} for 2 weeks prior to cardiac surgery. Treated patients had significantly higher plasma, atrial trabeculae and atrial mitochondrial CoQ_{10} levels (Rosenfeldt et al., 2005). There was symptomatic improvement, muscle levels of CoQ_{10} returned to normal, lipid storage resolved, ETC enzyme activities increased and muscle fiber apoptosis decreased after 8 months of CoQ_{10} treatment in two brothers with myopathic CoQ_{10} deficiency (Di Giovanni et al., 2001). Indirect evidence of cardiac uptake of CoQ_{10} is provided by the numerous underpowered controlled trials showing benefit in cardiac failure. A meta-analysis of these trials has recently been published (Sander et al., 2006). There is evidence of cardioprotection from the cardiomyopathy caused by chemotherapeutic agents and seven of eight controlled trials showing benefit of CoQ_{10} treatment

prior to cardiac surgery (Pepe et al., 2007). In idiopathic dilated cardiomyopathy there is level IV evidence of CoQ_{10} treatment benefit (Manzoli et al., 1990). In various muscle diseases there is evidence of improved exercise tolerance following CoQ_{10} treatment however it is difficult to exclude the possibility of a systemic effect of treatment independent of muscle uptake. Controlled trials providing Level II evidence of improved muscle strength have been reported in muscular dystrophy at CoQ_{10} doses of 100 mg/day (Folkers and Simonsen, 1995). Evidence of human brain effects of CoQ_{10} treatment comes from studies in two neurodegenerative disorders with mitochondrial OXPHOS abnormality, Huntington's disease (HD) and Parkinson's disease (PD) providing indirect evidence of brain penetration at high dosage in the human (Shults and Haas, 2005; Schapira, 2006; Beal, 2005).

5.3. CoQ_{10} deficiency in mitochondrial disease

Coenzyme Q_{10} supplementation might be expected to be particularly beneficial if there is secondary deficiency of CoQ_{10} in the mitochondrial disease patient. A subset of patients with primary mitochondrial disease, do have CoQ_{10} deficiency. As early as 1986 Ogasahara et al. reported low muscle CoQ_{10} levels in 5 patients with Kearns–Sayre syndrome (KSS) along with a lowering of the pyruvate and NADH oxidation in muscle and an improved CSF L/P ratio and protein concentration following treatment with 120–150 mg/d of CoQ_{10} (Ogasahara et al., 1986). However Zierz et al. found CoQ_{10} deficiency in muscle and plasma in only 1 of 5 patients with KSS (Zierz et al., 1989). In 1991 Matsuoka et al. reported that CoQ_{10} content in muscle mitochondria isolated from 25 patients with mitochondrial myopathies were significantly lower than controls (Matsuoka et al., 1991). There was marked variability from patient to patient and particularly in the group of KSS samples. Low serum CoQ_{10} levels compared to controls were noted in 8 patients with mitochondrial encephalomyopathy who underwent a double-blind crossover treatment trial (Chen et al., 1997). Montero et al. measured muscle CoQ_{10} concentrations in 34 pediatric patients and two adults classified according to Bernier criteria (Bernier et al., 2002) compared to pediatric controls (Montero et al., 2005). These authors found partial coenzyme Q_{10} deficiency in 6 of 13 patients classified as probable mitochondrial disease with NADH:cytochrome *c* reductase along with succinate:cytochrome *c* reductase reduced activities. Of 9 patients with a diagnosis of definite mitochondrial disease based on at least two major Bernier criteria only one (a neonate with mtDNA depletion) had a mild reduction of CoQ_{10}. Our experience in the UCSD Mitochondrial and Metabolic Disease Center suggests that muscle deficiency is seen in mitochondrial disease when patients are biopsied prior to CoQ_{10} treatment but low muscle CoQ_{10} is also seen in a variety of other conditions. In a series of muscle CoQ_{10} measurements by tandem mass spectroscopy in 107 consecutive muscle biopsy samples

from a mixture of pediatric and adult patients under investigation for unexplained muscle symptoms or multi-organ system disease, 16 samples (from patients ranging in age from 1 year to 72 years) had CoQ_{10} levels of 0.1 μg/mg protein or less (normal range 0.14–0.29 μg/mg protein). Four (25%) of these patients had definite primary genetic mitochondrial disease; 3 with mtDNA deletions and one adult Leigh disease patient with complex I deficiency. Low plasma CoQ_{10} levels were reported in the study group of 3 MELAS, 4 MERRF and 1 deletion patient (Chen et al., 1997).

It is important to note that plasma CoQ_{10} levels do not necessarily reflect tissue levels. This is particularly apparent in patients with primary CoQ_{10} deficiency where muscle and in some cases fibroblast levels must be measured for diagnosis (Quinzii et al., 2007).

5.4. Muscle CoQ_{10} deficiency in other conditions

Muscle CoQ_{10} deficiency is also found in conditions other than mitochondrial disease. Miles et al. reported low muscle CoQ_{10} (46%) compared to pediatric disease controls ($n = 21$) and an increased redox ratio of total ubiquinol/total ubiquinone in a group of 5 patients with muscular dystrophy. Respiratory chain enzyme activities in the three muscular dystrophy muscle samples studied were normal (Miles et al., 2005). A family with cerebellar ataxia and deficiency of CoQ_{10} has been found to have an aprataxin gene mutation as the underlying defect (Quinzii et al., 2005). Statin drugs have the potential to reduce CoQ_{10} synthesis as they inhibit 3-hydroxy-3-methylglutaryl coenzyme A reductase in the cholesterol synthesis pathway and thereby levels of farnesyl pyrophosphate which is required for CoQ_{10} synthesis. Studies in rats treated with statin drugs showed lower tissue CoQ levels (Low et al., 1992). Blood levels of CoQ_{10} are lowered by atorvostatin treatment in humans (Rundek et al., 2004) and 9 of 16 patients with statin drug-related myopathy had muscle levels greater than 1 standard deviation below the control mean (Lamperti et al., 2005).With the exception of statin treatment the mechanism of secondary coenzyme Q_{10} deficiency in patients with mitochondrial disease and other disorders is unknown. Decreased synthesis with or without increased mitochondrial turnover, increased CoQ_{10} degradation and the possibility that CoQ_{10} is lost from non-mitochondrial organelles and cellular membranes are considerations.

5.5. Effects of CoQ_{10} deficiency on mitochondrial function

The expected effect of mitochondrial CoQ_{10} deficiency on mitochondrial electron transport is a reduction of electron flux through the CoQ_{10} site. A characteristic signature of deficiency is produced. There is a low level of oxygen flux in polarographic assays of site 1- and site 2-dependent substrates where electrons must traverse CoQ_{10} but normal activity of cytochrome oxidase, which is distal to CoQ_{10}.

Spectrophotometric assay of individual electron transport complexes will produce normal or elevated results with low activities in assays which bridge the CoQ_{10} site (NADH:cytochrome *c* reductase and succinate:cytochrome *c* reductase). There may be a compensatory increase in mitochondrial content determined by histochemistry, electron microscopy or by assay of citrate synthetase activity. This pattern of electron transport dysfunction has been found in muscle, fibroblast and lymphoblast studies from patients with primary CoQ_{10} deficiency (Rotig et al., 2000; Ogasahara et al., 1989; Musumeci et al., 2001; Salviati et al., 2005; Artuch et al., 2006; Boitier et al., 1998) but there is little information on electron transport chain function due to secondary deficiency of CoQ_{10} in mitochondrial disease or other disorders.

Putative roles for coenzyme Q_{10} supplementation include: enhancement of electron transport and ATP production, antioxidant protection, a beneficial alteration in redox signaling, and stabilization of the mitochondrial permeability transition pore protecting against apoptotic cell loss.

6. Effects of CoQ_{10} treatment

There is evidence of benefit from CoQ_{10} supplementation in animal and human studies. Life long supplementation with low dose (0.7 mg/kg) in the rat resulted in higher plasma levels of coenzyme Q_9, coenzyme Q_{10}, α-tocopherol and retinol. Treated aged rats showed higher levels of polyunsaturated fatty acids and less DNA strand breaks in blood lymphocytes (Quiles et al., 2005). In the cardiac surgery study mentioned above there were documented higher cardiac coenzyme Q_{10} levels and improved contractility with protection against anoxic injury in isolated cardiac muscle suggesting a potential benefit despite the lack of discernable benefit in post-operative measures of cardiac function in the treated patients (Rosenfeldt et al., 2005). Level III evidence of cardiac benefit was provided by an open label treatment trial over 47 months in 10 genetically confirmed Friedreich's ataxia patients compared to 77 control subjects. Combined CoQ_{10} (400 mg/d) and vitamin E (2100 IU/d) treatment produced a significant improvement in cardiac and skeletal muscle bioenergetics. Echocardiographic data revealed significantly increased fractional shortening on treatment and although neurological disease progressed the changes in total International Cooperative Ataxia Rating Scale and kinetic scores over the trial period were better than predicted for 7 of the 10 patients (Hart et al., 2005). Other studies in neurodegenerative diseases provide Level I evidence of CoQ_{10} efficacy. In HD brain lactate levels were lower after treatment with 360 mg/day (Koroshetz et al., 1997) although a subsequent large placebo-controlled study at a dose of 600 mg/day was unable to show any effect on the Total Functional Capacity outcome measure (Huntington's Study Group, 2001). In PD a slowing of the rate of decline in the Unified Parkinson Disease Rating Scale (UDPRS) was demonstrated at a CoQ_{10} dose of 1200 mg/day (plus vitamin E at 1200 IU) with a trend to improvement at lower doses (Shults et al., 2002). This treatment trial reached Level 1 evidence criteria. Safety and tolerability of CoQ_{10} doses up to 3000 mg/day have been demonstrated in PD (Shults et al., 2004) and amyotrophic lateral sclerosis (ALS) (Ferrante et al., 2005). In HD doses up to 3600 mg/day have been shown to be well tolerated and safe (Huntington Study Group, 2005). In all three of these dose ranging trials however it was noted that blood levels plateaued at doses above 2400 mg/day. The realization of the need for high dosing levels and the encouraging results to date in PD and HD has lead to the planned start of two major NIH funded placebo-controlled treatment trials of high dose CoQ_{10} treatment in PD and HD with 600 and 608 patients, respectively.

7. Data on the use of CoQ_{10} in mitochondrial disease

7.1. Exercise testing in mitochondrial myopathies

Exercise testing has the advantage of providing numerical data with which to gauge treatment effects. It should be realized however that exercise protocols test more than just muscle power and endurance. They tend also to assess whole body cardiovascular performance.

In 1998 the use of standardized exercise testing in mitochondrial myopathy patients coupled with the measurement of the lactate/pyruvate ratio was reported by Chan et al. (1998). These investigators utilized a standardized bicycle ergometer protocol with serial blood lactate and pyruvate measurements at 4, 8, 12 and 16 min after the start of exercise and 9 and 29 min after exercise cessation. The protocol involved a 15-min rest period followed by exercise at 30 W with continuous ECG monitoring. Exercise was increased by 10 W every 2 min until a maximum of 100 W was reached or exhaustion compelled the subject to stop. Nine patients were reported with ragged red fiber disease and opthalmoplegia, 2 had KSS. Five of the nine had mitochondrial DNA deletions. The findings were that all nine patients showed pathological lactate/pyruvate (L/P) ratios on exercise and following 6 months of 150 mg of CoQ_{10} treatment daily L/P ratios returned to normal in three. Looking at lactate measurements alone abnormalities were found in only 6 of the 9 treated patients with a decrease to the normal range after 6 months of treatment in one of these. The authors point out that the L/P ratio is a measure of the redox state and mirrors the cellular $NADH/NAD^+$ ratio. Although this was an open-label study, ergometer testing and lactate/pyruvate measurements are relatively objective tests of response efficacy.

Exercise testing with 31P-MRS studies have been reported in three open-label studies totaling 16 subjects with mitochondrial disease with improvement found in most patients (Table 1) (Gold et al., 1996; Bendahan et al., 1992; Barbiroli et al., 1997).

Table 1
Studies of coenzyme Q_{10} in OXPHOS disease excluding Level V evidence

Disorder	Treatment duration	Study design	Dose	Number of Subjects	Outcome measure (s)	Outcome: +/− or NC (no change)	Author(Reference)	Year	Evidence level
MitoEncMy	3 months	DBX	160 mg/d	8	Global MRC index score	+	Chen et al. (1997)	1997	II
CPEO, KSS MERRF MitoMy	3 months	DB	2 mg/kg/d	16	Bicycle ergometer lactate,neuro exam	+NC	Bresolin et al. (1990)	1990	II
CPEO	9 months	DBX	100 mg/d	17 entered 7 completed	Various including muscle power	NC	Muller et al. (1990)	1990	II
MELAS	3 years	OL	150 mg/d	28	Insulin secretory response & C-peptide	+	Suzuki et al. (1998)	1998	III
MitoCytop	6 months	OL	150 mg/d	6	Muscle & Brain 31P-MRS	+	Barbiroli et al. (1997)	1997	III
MELAS	2 weeks	OL	200 mg/d	2	Bicycle ergometer L+P, muscle O2 by NIRS	+ in 1	Abe et al. (1999)	1999	IV
MitoEncMy	6 months	OL	150 mg/d	9	Bicycle ergometer L/P ratio	+ in 6 of 9	Chan et al. (1998)	1998	IV
MitoEncMy	6 months	OL	150 mg/d	8	Muscle 31P-MRS	+ in 1 of 8	Gold et al. (1996)	1996	IV
MitoEncMy	10 months	OL	150 mg/d	2	Bicycle ergometer lactate and muscle 31P-MRS	+	Bendahan et al. (1992)	1992	IV
CPEO, KSS MERRFMitoMy	6 months	OL	2 mg/kg/d	44	Bicycle ergometer lactate,MRC Index Score	+NC	Bresolin et al. (1990)	1990	IV
KSS		OL	150 mg/d	2	Muscle strength & fatigue, ADL, weight	+	Desnuelle et al. (1989)	1989	IV
KSS, CPEO + MitoMy	1 year	OL	120 mg/d	7	Exercise lactate & pyruvate	+	Bresolin et al. (1988)	1988	IV
KSS	1.2 years	OL	120–150 mg/d	5	Bicycle ergometer L/P, CSF protein and L/P, ECG, Neuro exam, ADL	+	Ogasahara et al. (1986)	1986	IV

MitoEncMy, mitochondrial encephalomyopathy; MitoMy, mitochondrial myopathy; MitoCytop, mitochondrial cytopathy; KSS, Kearns–Sayre syndrome; CPEO, chronic progressive opthalmoplegia; MELAS, mitochondrial encephalomyopathy with lactic acidosis and stroke-like episodes; DBX, double-blind crossover; DB, double-blind; OL, open label; NIRS, near-infrared spectra; ADL, activities of daily living.

7.2. Trials of coenzyme Q_{10} in mitochondrial disease

A recent Cochran review of treatment for mitochondrial disorders amended March 6, 2006 evaluated 677 abstracts from Medline and various registries of clinical trials up to 2003 identifying only 11 treatment studies of which six met criteria for randomized controlled treatment trials and only two were trials of CoQ_{10} as the sole treatment variable. Excluded studies included case reports, open-label trials and retrospective studies (Chinnery et al., 2006). These two included controlled trials were both studies using low doses of CoQ_{10} in small numbers of subjects, 8 and 17, respectively, but they do meet Level II evidence criteria (Muller et al., 1990; Chen et al., 1997). The study by Chen et al. published in 1997 was a randomized placebo controlled double-blind crossover study of 8 subjects treated with 160 mg/day of CoQ_{10} for 3 months or placebo for 1 month with a 1-month washout period (Chen et al., 1997). All study subjects had genetic mitochondrial disease. Four subjects had the syndrome of Myoclonic Epilepsy with Ragged-Red Fibers (MERRF), 3 had Mitochondrial Encephalomyopathy with Lactic Acidosis and Stroke-like episodes (MELAS) and 1 subject had Chronic Progressive External Opthalmoplegia (CPEO) with myopathy. Only in the global MRC index score was statistically significant improvement found ($p < 0.05$). However treatment benefit trends were noted in several study parameters. These included alleviation of fatigability in daily activities, enhanced endurance to muscle exercise and an increase in global muscle strength scored by Medical Research Council scale. Also the elevation in serum lactate and pyruvate levels after exercise was decreased. The second study was reported by Muller et al. (1990). This was a double-blind crossover study of 17 subjects (6 men and 11 women) with CPEO reported only in abstract. Subjects were treated with placebo or 100 mg/day of CoQ_{10} for 9 months on each treatment. Seven subjects were reported to have completed the trial with 'no response' on the parameters tested; muscle power and muscle MR imaging and spectroscopy, exercise ECG, nerve conduction studies and electromyography, evoked potentials and serum lactate (Muller et al., 1990). The authors of the Cochran report were unable to provide a meta-analysis because of the limited size of treatment studies, heterogeneity of patient groups and the use of different study end points. A third study also meets Level II evidence criteria. A small double-blind study of 16 'responder' patients with mitochondrial myopathy treated for 3 months at a dose of 2 mg/kg could not demonstrate symptomatic improvement although post-exercise lactate levels were improved (Bresolin et al., 1990). All of these Level II studies used low doses of CoQ_{10} by today's treatment standards.

The majority of the evidence supporting efficacy of CoQ_{10} treatment lies in Level III studies and case reports (Table 1). The earliest and quite convincing study was an open label Level IV study of CoQ_{10} 120–150 mg/day treatment over 1.2 years in 5 patients with KSS. Objective parameters of CSF protein and the L/P ratio were improved along with significant improvements in ECG abnormalities. Less objective parameters of ADL and bicycle ergometer exercise lactate plus pyruvate and L/P ratios were also improved (Ogasahara et al., 1986). An open-label multicenter trial of CoQ_{10} treatment for 6 months in 44 subjects with mitochondrial myopathy at the low dose of 2 mg/kg/day allowed selection of 16 subjects who seemed to be responders with a 25% or greater decrease in post-exercise lactate levels. These 16 subjects then entered a blinded placebo-controlled Level II trial and were treated for a further 3 months at the same low dosage – with maintenance of improvement in post-exercise lactate in the treated group but a tendency for the placebo group to deteriorate (Bresolin et al., 1990). Two level III studies by are listed in Table 1. The long-term 3-year study of 28 MELAS subjects treated with 150 mg/d of CoQ_{10} by Suzuki et al. found improvements in insulin secretory response and C-peptide levels (Suzuki et al., 1998). Barbiroli et al. studied 6 patients with mitochondrial cytopathy treated for 6 months with 150 mg/d of CoQ_{10} compared muscle and brain 31P-MRS to controls and reported improvement to control levels in the occipital lobe phosphocreatinine ratio, the calculated ADP levels and relative rate of ATP synthesis. In the muscle measures of mitochondrial function improved (Barbiroli et al., 1997). Eight Level IV studies of case series are summarized in Table 1. It is notable that all reported Level II through IV studies in mitochondrial subjects found some evidence of CoQ_{10} efficacy except for the small study by Muller et al. and none reported any major side effects apart from a single subject who developed hives on starting treatment (Table 1). The most common minor complaint in studies of CoQ_{10} treatment is gastrointestinal symptomatology.

Three publications report effects of CoQ_{10} in combination with other treatments. The study by Matthews et al. administered a cocktail of CoQ_{10} 300 mg/d, vitamin K_3 60 mg/d, ascorbate 2 g/d, thiamin 100 mg/d, riboflavin 25 mg/d and niacin 200 mg/d for 2 cycles of 2 months on-treatment compared to 2 months off-treatment to a heterogeneous collection of 16 mitochondrial disease patients (Matthews et al., 1993). The authors concluded in this short open treatment trial that there was no demonstrable benefit. Peterson reported on the long-term open-label treatment of 16 patients with 'well-characterized mitochondrial disease'. The treatment was a low dose of CoQ_{10} (60–120 mg/d), vitamin K_3 20–60 mg/d, ascorbate 2 g/d and methylprednisolone 2–16 mg alternate days (Peterson, 1995). Based on outcome measures of clinical evaluation, 31P-NMR spectroscopy and near-infrared spectroscopy the authors concluded that 'treated patients appeared to survive longer with less functional disability than typically seen in clinical practice'. Rodriquez et al. reported treatment benefit in a Level II randomized, double-blind, placebo controlled, crossover study of 17 patients, 16 of whom completed the study. The patient group consisted of MELAS (3), mtDNA deletions (4), CPEO (3), KSS (1)

and 9 subjects with other mitochondrial diseases. Patients were treated with placebo and treatment for 2 months each with a 5 week washout period between. Daily treatment consisted of 3 g of creatine monohydrate, 300 of lipoic acid and 120 mg of CoQ_{10}. Treatment produced lower resting lactate levels, lower urinary 8-isoprostanes, and an attenuation in the decline in peak ankle dorsiflexion group with an increase in fat-free mass seen only in the MELAS subjects (Rodriguez et al., 2007).

8. Conclusions

So where does this leave us? Is coenzyme Q_{10} helpful in the treatment of mitochondrial disease? The preponderance of the evidence suggests some benefit without major toxicity. However the majority of this evidence comes from Level III and IV open label studies. Only three studies achieve Level II significance, two showing benefit and one without effect. Most studies use low doses of CoQ_{10} without information on blood or tissue levels. Because of the great difficulty of studying this heterogeneous population and historically poor funding in the area of clinical trials in mitochondrial disease the evidence base to answer the question of CoQ_{10} efficacy is currently poor. An FDA sponsored Phase III double-blind placebo-controlled cross-over trial started recruiting subjects in January 2007 for a 2-year study in 50 mitochondrial disease patients aged 12 months to 17 years. The primary outcome measures are gross motor function and home quality of life (clinical trials.gov). However at this time the evidence base consists of only 3 blinded Level II studies totaling 38 patients who completed the trials. There are in addition 10 open-label studies two of which provide Level III evidence with included control comparisons. The total number of subjects in these trials is 113. There remains in addition to the published data considerable anecdotal supportive information from single case reports and personal experience of clinicians treating mitochondrial disease patients. There is Level I evidence of some benefit from coenzyme Q_{10} treatment in Parkinson's disease where there is associated mitochondrial dysfunction and measured lowered coenzyme Q_{10} tissue levels. In Friedreich's ataxia where mitochondrial function is impaired, Level III evidence suggests a treatment benefit. Larger scale trials are underway in a number of other neurodegenerative diseases including ALS and Huntingdon's disease. These diseases share a common feature – secondary mitochondrial dysfunction. In the case of primary genetic mitochondrial disease it is worth stating that absence of clinical trial data does not equate to a lack of efficacy. The practice of evidence-based medicine requires clinicians to use the best available scientific data to make treatment decisions even when this is Level IV and Level V information. At present despite the lack of adequate clinical trials in mitochondrial disease the majority of practitioners working with mitochondrial disease patients treat their patients with coenzyme Q_{10} supplementation. As dosage and expense are considerations, highly bioavailable preparations are preferred.

Acknowledgment

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Coenzyme Q treatment of neurodegenerative diseases of aging

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Abstract

The etiology of several neurodegenerative disorders is thought to involve impaired mitochondrial function and oxidative stress. Coenzyme Q-10 (CoQ_{10}) acts both as an antioxidant and as an electron acceptor at the level of the mitochondria. In several animal models of neurodegenerative diseases including amyotrophic lateral sclerosis, Huntington's disease, and Parkinson's disease, CoQ_{10} has shown beneficial effects. Based on its biochemical properties and the effects in animal models, several clinical trials evaluating CoQ_{10} have been undertaken in many neurodegenerative diseases. CoQ_{10} appears to be safe and well tolerated, and several efficacy trials are planned.
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1. Introduction

There are several lines of evidence implicating oxidative stress and impaired mitochondrial function in the neurodegenerative processes of amyotrophic lateral sclerosis (ALS), Huntington's disease (HD), and Parkinson's disease (PD) (Beal, 2005). As presented in detail in prior articles in this edition, Coenzyme Q-10 (CoQ_{10}, ubiquinone), acts both at the level of the mitochondria as an electron acceptor for complexes I and II of the electron transport chain and as an antioxidant (Ernster and Dallner, 1995). Based on these properties and the experimental findings that CoQ_{10} protects against neuronal loss in several animal models of neurodegeneration, clinical trials investigating the ability of CoQ_{10} to slow disease progression have been considered for many of the neurodegenerative diseases. To date, several pilot, dosage finding and safety studies have been conducted. Definitive efficacy studies have not yet been completed. The clinical trials evaluating CoQ_{10} in ALS, HD, PD, and other neurodegenerative diseases are reviewed (Table 1) and critical issues inherent to these trials are discussed.

2. Amyotrophic lateral sclerosis

2.1. Clinical background

Amyotrophic lateral sclerosis (ALS) is a neuromuscular disorder characterized by degeneration of the motor neurons in the anterior horns of the spinal cord, brainstem, and motor cortex with resultant progressive muscle weakness, atrophy, and spasticity. The incidence of ALS is approximately 1 to 2 cases per 100,000 per year with onset typically in the 60s and survival of 3–5 years (Rowland and Shneider, 2001; Sorenson et al., 2002). While the majority of cases are sporadic, approximately 10% are familial, a subset of which are due to mutations in the gene coding for the antioxidant enzyme Cu/Zn superoxide dismutase (SOD1) (Rosen et al., 1993). Although the etiology of ALS is not known, several lines of evidence suggest increased oxidative damage in both familial and sporadic ALS (Beal et al., 1997; Ferrante et al., 1997). Consistent with such findings, the antioxidant enzyme glutathione peroxidase has been shown to be reduced in brain regions

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Table 1
Clinical trials of CoQ$_{10}$

	Dosage	Subjects	Outcome measures	Conclusion	Reference
ALS					
	1200, 1800, 2400, 3000 mg/d[a]	31	Safety, tolerability, CoQ$_{10}$ levels	3000 mg/d safe, well tolerated; plasma levels plateau between 2400 and 3000 mg/d	(Ferrante et al., 2005)
	1800, 2700 mg/d; placebo	115	Change in ALSFRS-R	Ongoing	(Levy et al., 2006)
HD					
	360 mg/d	18	Change in brain lactate levels (NMR spectroscopy)	CoQ$_{10}$ decreases cortical lactate levels	(Koroshetz et al., 1997)
	600 mg/d	10	Safety, tolerability, efficacy	600 mg/d safe, well tolerated	(Feigin et al., 1996)
	600 mg/d; placebo[b]	347	Efficacy (change in TFC from baseline to 30-month)	No significant difference in TFC as compared to placebo	(Huntington Study Group, 2001)
	1200, 2400, 3600 mg/d	28 (20 HD; 8 healthy)	Safety, tolerability, CoQ$_{10}$ levels	Safe, well tolerated; plasma levels plateau between 2400 and 3600 mg/d	(Hyson and Huntington Study Group, 2005)
	2400 mg/d; placebo	608	Change in TFC from baseline to 5-year	Planned	ME Cudkowicz, Principal Investigator
PD					
	400, 600, 800 mg/d[a]	15	Safety, tolerability, plasma CoQ$_{10}$ levels, mitochondrial function, UPDRS	Safe, well tolerated	(Shults et al., 1998)
	300, 600, 1200 mg/d; placebo[a]	80	Change in UPDRS from baseline to 16 months or need for symptomatic therapy; CoQ$_{10}$ levels, mitochondrial function	Significant linear trend between dosage and mean change in UPDRS	(Shults et al., 2002)
	360 mg/d; placebo	28	UPDRS, color vision	Decrease in UPDRS in CoQ$_{10}$ compared to baseline; not different than placebo	(Muller et al., 2003)
	1000, 1500 mg/d	12	Motor tasks, Part II UPDRS	Improvement in motor tasks compared to baseline	(Horstink and van Engelen, 2003)
	1200, 1800, 2400, 3000 mg/d[a]	17	Safety, tolerability, plasma CoQ$_{10}$ levels, UPDRS	Safe, well tolerated; plasma levels plateau between 2400 and 3000 mg/d; no change in motor UPDRS	(Shults et al., 2004)
	2400 mg/d, placebo[a]	71	UPDRS from baseline to 12 months or need for symptomatic therapy	Not found to be futile based on prespecified futility threshold	(The NINDS NET-PD Investigators, 2007)
	1200 and 2400 mg/d, placebo[a]	600	Change in total UPDRS from baseline to 16 months or need for symptomatic therapy	Planned	MF Beal, Principal Investigator
AD					
	2400 mg/d; vitamins E, C, and lipoic acid; placebo	75	Safety, tolerability; CSF biomarkers	Ongoing	NCT00117403[c]
PSP					
	5 mg/kg/d; placebo	30	Safety, tolerability; imaging; motor, cognitive assessments	Ongoing	NCT00328874[c]
	Not specified; placebo	60	Functional decline, safety, quality of life	Ongoing	NCT00382824[c]

[a] Formulation included vitamin E.
[b] Study was a 2 × 2 factorial design (CoQ$_{10}$, remacemide, placebo).
[c] ClinicalTrials.gov Identifier.

affected by ALS compared to both unaffected brain regions and brain regions from unaffected controls (Przedborski et al., 1996). In addition, mitochondria have been implicated in the pathophysiology of ALS. Impaired mitochondrial complex I function (Wiedemann et al., 1998) as well as abnormal mitochondrial morphology (Siklos et al., 1996) have been shown in skeletal muscle of subjects with ALS. Although it is not entirely known if such alterations are primary defects or changes secondary to denervation, similar impairments in mitochondrial function were not noted in patients with spinal muscular atrophy, another disorder characterized by motor neuron degeneration

(Wiedemann et al., 1998), and such morphologic changes were more pronounced than those observed in other neuropathies characterized by denervation (Wiedemann et al., 1998). In addition, mitochondrial gene mutations cause motor neuron disease (Pasinelli and Brown, 2006). Interestingly, mutant SOD1 may act at the level of the mitochondria, forming aggregates and consequently causing apoptotic cell death (Pasinelli et al., 2004; Pasinelli and Brown, 2006).

2.2. Role of CoQ10

Serum CoQ_{10} levels and the CoQ_{10}/cholesterol ratio have been shown to be similar in ALS subjects and controls (Molina et al., 2000), yet higher plasma levels of oxidized CoQ_{10} have been found in patients with sporadic ALS consistent with oxidative stress (Sohmiya et al., 2005). As there is evidence of both mitochondrial dysfunction and oxidative stress in the pathogenesis of ALS, CoQ_{10} has been examined as a therapeutic approach.

Studies in an animal model of ALS have suggested that CoQ_{10} may be effective in the treatment of ALS. In a transgenic mouse model with a SOD1 mutation, supplementation with 200 mg/kg of CoQ_{10} increased survival, suggesting a potential therapeutic role for CoQ_{10} in patients (Matthews et al., 1998). Recently, a systematic review of candidate agents was conducted for ALS, and CoQ_{10} was viewed as one of 20 priority agents for investigation in clinical trials (Traynor et al., 2006).

2.3. Clinical trials

A tolerability study of CoQ_{10} in ALS has demonstrated that dosages of 3000 mg/d are safe and well tolerated for up to 8 months (Ferrante et al., 2005). In this open label study, 31 subjects received CoQ_{10} (600 mg wafer with 300 IU vitamin E) on a monthly dosage escalation schedule of 1200 mg daily, then 1800 mg, followed by 2400 mg, and finally 3000 mg daily for a period of 5-month. Tolerability was assessed by subjects' ability to complete the trial according to the dosing regimen without any dosage reductions or suspensions. Safety was evaluated by laboratory tests, clinical findings, and adverse events. In addition, to look for evidence of efficacy, rates of change in maximum voluntary isometric contraction, vital capacity, and ALS Functional Rating Scale (ALSFRS-R) were compared to historical control data. Twenty-five subjects were on study drug at the completion of the study, 24 of whom were on the maximal dosage of 3000 mg daily. The frequencies of adverse events were similar to those in the historical placebo group and included headache, peripheral edema, rash, and urinary tract infection each in 16.1% of subjects, as well as abdominal pain, constipation, diarrhea, arthralgia, and infection in 12.9% of subjects. Plasma levels of CoQ_{10} were obtained prior to each dosage escalation, and levels were increased compared to baseline at all dosages

evaluated except 1200 mg/day. Of note, no difference in plasma levels was noted between 2400 and 3000 mg.

A randomized, placebo controlled, double blind, multi-center trial of CoQ_{10} is currently underway (QALS study) (Levy et al., 2006). During the first stage of this study, 35 subjects were randomized to each of three groups: CoQ_{10} 1800 mg/d, CoQ_{10} 2700 mg/d, or placebo. The preferred dosage will be selected using statistical procedures, and this dosage then will be compared to placebo to evaluate for preliminary signs of efficacy using a futility design. In addition to the 35 subjects from each arm in Stage 1, 80 subjects will be randomized in a 1:1 ratio, resulting in 75 subjects per arm. The primary futility threshold is defined as a decrease in the ALSFRS-R by at least 20% compared to placebo. This study design is efficient as it employs a small sample size to identify a futile dosage and incorporates the data from the dosage selection stage in the efficacy stage. Stage 1 has completed enrollment.

3. Huntington's disease

3.1. Clinical background

Huntington's disease is an autosomal dominant disorder due to a mutation in exon 1 of the huntingtin gene on chromosome 4 which results in an expanded CAG repeat (The Huntington's Disease Collaborative Research Group, 1993). Clinically, this disorder is characterized by chorea, behavioral and psychiatric disturbances, and cognitive dysfunction. The prevalence of HD is 3–7 per 100,000, and the annual incidence is 0.2–0.7 per 100,000 (Cardoso et al., 2006). Disease onset is typically in the fourth to fifth decades and is inversely correlated to repeat length with an earlier onset observed in those with a greater CAG repeat number (Duyao et al., 1993). Disease duration is on average 15–20 years. Pathologically, there is progressive degeneration of striatal GABAergic medium spiny neurons and cortical projection neurons with relative sparing of striatal interneurons.

The mechanism by which mutations in huntingtin cause HD remains elusive, yet evidence from animal models and clinical specimens indicates a role of oxidative stress and impaired mitochondrial function. Lesions produced by injections of the mitochondrial complex II inhibitors malonate and 3-nitropropionic acid cause a pattern of striatal pathology in rats which is reminiscent of that observed in HD patients (Beal et al., 1993) and choreiform movements in primates similar to the involuntary movements seen clinically (Brouillet et al., 1995).

A role for the contribution of impaired mitochondrial function is further supported by post-mortem studies of brains from HD patients as well as biochemical and imaging studies. As compared to controls, there is a decrease in complexes II, III, and IV in the caudate and putamen of HD patients (Mann et al., 1990; Gu et al., 1996; Browne et al., 1997). In addition, levels of 8-hydroxydeoxyguanosine, a measure of oxidative DNA damage, are increased

in the caudate in post-mortem samples (Browne et al., 1997). Mitochondria isolated from individuals with HD show abnormal calcium homeostasis (Panov et al., 2002). Further evidence of abnormalities of mitochondrial function comes from analysis of cerebrospinal fluid. The lactate:pyruvate ratio, an indicator of impaired energy metabolism, is elevated in HD patients (Koroshetz et al., 1997). In addition, localized NMR spectroscopy studies demonstrate elevated lactate levels in the occipital cortex and basal ganglia of HD patients (Jenkins et al., 1993,1998; Koroshetz et al., 1997).

3.2. Role of CoQ_{10}

CoQ_{10} has been shown to be effective in decreasing lesions produced by the toxins which inhibit complex II (Beal et al., 1994; Matthews et al., 1998) and in preventing ATP depletion and lactate increases (Beal et al., 1994). CoQ_{10} has also been shown to prolong survival and delay the onset of motor deficits in a transgenic mouse model of HD (Ferrante et al., 2002).

3.3. Clinical trials

As mentioned above, HD patients have elevated brain lactate levels. CoQ_{10} administration at a dosage of 360 mg/d for 2–8 weeks was associated with a decrease in lactate levels in the occipital cortex in 15/18 subjects (Koroshetz et al., 1997). Following discontinuation of CoQ_{10}, imaging showed a return to baseline values indicating this effect was due to CoQ_{10}. These intriguing results regarding the ability of CoQ_{10} to alter cortical lactate levels support the therapeutic potential of CoQ_{10} for a treatment in HD.

A 6-month, open label, pilot trial has assessed the tolerability of CoQ_{10} in HD subjects (Feigin et al., 1996). Ten subjects with symptomatic HD received CoQ_{10} at a dosage of 600 mg a day divided as 200 mg three times daily. At baseline, 3, and 6 months, subjects were evaluated using the Huntington's Disease Rating Scale, the HD Functional Capacity Scale, and neuropsychological tests including Trailmaking A and B, Verbal Fluency, Symbol Digit Modalities, Stroop Interference, and Paired Associate Learning. All subjects completed the study, and two subjects increased their own dosages up to 1000 and 1200 mg and remained on these increased dosages for the duration of the study. Adverse events were mild and included heartburn, fatigue, headache, and increased involuntary movements. No subjects required dosage reduction or drug discontinuation. When comparing results on the motor and functional scales from baseline to 6 months, there was no significant effect of CoQ_{10} on these measures. However, the study was small and not powered to detect such effects.

CoQ_{10} was further evaluated in a randomized, double blind, placebo controlled, parallel group clinical trial (CARE HD) in which subjects received CoQ_{10} 300 mg twice a day, the non-competitive NMDA receptor antagonist

remacemide 200 mg three times a day, or placebo in a 2×2 factorial design (Huntington Study Group, 2001). A total of 347 subjects with early HD were enrolled in this 30-month study which was aimed at determining if CoQ_{10} slowed the functional decline typically observed in HD. The primary outcome measure was the change in the Total Functional Capacity (TFC) Scale between baseline and 30-month. Additional measures of efficacy which were evaluated included the total motor score and subscales of the Unified HD Rating Scale (UHDRS), Stroop Test, Independence Scale, Hopkins Verbal Learning Test, Brief Test of Attention, and Conditional Associative Learning Test as well as additional neuropsychological tests. The study was powered to detect a 40% slowing in the decline in the TFC scale.

This dosage of CoQ_{10} was well tolerated with only 4 subjects discontinuing study drug prior to the completion of the study. Of the reported adverse events, only "stomach upset" was more common amongst subjects receiving CoQ_{10}. At 30-month, no significant difference in functional decline as measured by change in TFC score from baseline was found between treatment arms. When looking at all subjects who received CoQ_{10} versus those who did not receive CoQ_{10} across the treatment arms, a 13% slower decline in the TFC was noted. Additional secondary analyses demonstrated encouraging results on functional and behavioral assessments as well as some of the cognitive evaluations (Stroop and Brief Test of Attention). The lack of statistical efficacy in this trial may be that the study was underpowered to detect the observed effect size or that the dosage evaluated was insufficient. Of note, examination of the graphical data suggest the beginnings of a separation of the CoQ_{10} arm from the placebo arm on several of the outcome measures at about 11 months raising the possibility that longer treatment and follow-up may be necessary to detect a change.

A small pilot trial (Pre-2CARE) was conducted to investigate the tolerability and plasma levels achieved with various dosages of CoQ_{10} (Huntington Study Group, 2005). Twenty HD subjects and 8 healthy controls were enrolled in this open label, dosage-ranging study. The dosage of CoQ_{10} was initially 1200 mg daily and was increased by 1200 mg every 4 weeks up to 3600 mg daily which was continued for a total of 12 weeks. Five subjects did not achieve the maximal dosage, and 6 subjects withdrew from the study prior to completion. The reasons for early termination were gastrointestinal symptoms (3), exacerbation of HD symptoms (2), and fall (1). The most frequently reported adverse event was gastrointestinal complaints. Blood levels of CoQ_{10} were found to increase with increasing dosages although the 3600 mg dosage did not provide a further significant increase.

Currently, a Phase III trial of CoQ_{10} is planned to determine if CoQ_{10} can slow the progression of HD. Nearly 600 subjects will be randomized to 2400 mg CoQ_{10} or placebo in a 1:1 ratio and followed for 5 years. Subjects will be evaluated at baseline, 1 and 3 months, and then every 6-month.

Evaluations will include the UHDRS, and the primary outcome measure will be the change in TFC over 5 years.

4. Parkinson's disease

4.1. Clinical background

Parkinson's Disease (PD) is a neurodegenerative disorders characterized clinically by tremor, rigidity, bradykinesia, and postural instability (Lang and Lozano, 1998). In addition to the motor manifestations which are presumed to be due to nigral cell loss and secondary striatal dopamine depletion, there are non-motor features including cognitive and autonomic dysfunction. The prevalence of PD is estimated to be 0.3% of the population and 1% of those over 60 years of age, and the incidence rate 8–18 per 100,000 person-years (de Lau and Breteler, 2006). While the majority of cases are idiopathic, several genetic mutations which cause parkinsonism have been identified including mutations in *PINK1* (Valente et al., 2004) and *DJ-1* (Bonifati et al., 2003). Since PINK1 is a mitochondrial protein kinase and DJ-1 is involved in oxidative stress, these particular mutations are interesting in view of the processes which may underlie the pathogenesis of PD (Greenamyre and Hastings, 2004).

Mitochondrial dysfunction and oxidative stress are thought be important in the pathogenesis of PD, and there are several lines of supporting evidence. The initial hypothesis that mitochondrial complex I deficiency may be involved in the etiology of PD came from the findings that the mitochondrial complex I inhibitor MPTP causes a clinical syndrome indistinguishable from PD and selective dopaminergic cell loss in the substantia nigra (SN) (Langston et al., 1983). Subsequently, complex I activity in PD patients was assessed and found to be significantly reduced in platelet mitochondria in PD subjects as compared with controls (Parker et al., 1989; Haas et al., 1995) as well as in the SN, but not other regions of the brain, from PD patients (Schapira et al., 1990a,b). Decreases in complex II/III activity have also been noted in platelet mitochondria (Haas et al., 1995).

4.2. Role of CoQ₁₀

Based on the findings of decreased activity of complexes I and II/III in the electron transport chain and the fact that CoQ_{10} is the electron acceptor for these complexes, levels of CoQ_{10} were measured in mitochondria from PD subjects (Shults et al., 1997). CoQ_{10} levels were found to be significantly lower in PD patients than in age-matched controls and to correlate with activity of complex I and II/III. In addition, the percent of CoQ_{10} which is in its oxidized form is elevated in PD patients further supporting a role of oxidative stress in the pathogenesis of this disease (Sohmiya et al., 2004). These findings support the idea that increasing CoQ_{10} levels could be of therapeutic benefit. Animal model data support this hypothesis as CoQ_{10} (200 mg/kg/d) was

able to partially protect against the decrease in striatal dopamine concentration and tyrosine hydroxylase fiber density associated with MPTP administration in mice (Beal et al., 1998).

4.3. Clinical trials

A pilot study in 15 PD subjects was conducted to assess tolerability and absorption of CoQ_{10} (Shults et al., 1998). Subjects, 5 per dosage cohort, received CoQ_{10} 200 mg with Vitamin E 400 IU twice daily, three times daily, or four times daily for 30 days. Tolerability, plasma CoQ_{10} levels, platelet mitochondrial function, and the motor subpart of the Unified Parkinson Disease Rating Scale (UPDRS) were evaluated. There were no adverse events reported by the subjects. Minor abnormalities in urinalyses of unclear clinical significance were noted in 2 of the 5 subjects receiving the highest dosage. CoQ_{10} levels were increased at all dosages as compared with baseline, yet differences between dosages were not significant. There was a trend toward increased complex I activity. There was no evidence of a symptomatic effect of CoQ_{10} as there was no change in motor function as measured by the UPDRS during the course of this study.

Subsequently, a dosage ranging pilot study evaluated the safety and tolerability of CoQ_{10} as well as the ability of CoQ_{10} to slow functional decline in PD (QE2) (Shults et al., 2002). Eighty subjects with early PD not yet requiring symptomatic therapy were randomly assigned to receive CoQ_{10} at a dosage of 300, 600, or 1200 mg or placebo. All subjects also received vitamin E 1200 IU daily. Subjects were evaluated at 1, 4, 8, 12, and 16 months after baseline and followed until 16 months or until the time at which symptomatic therapy was required, whichever came first. Plasma CoQ_{10} levels, platelet mitochondrial function, safety, tolerability, and compliance were assessed. The primary efficacy outcome measure was defined as the change in UPDRS from baseline to 16 months or the time at which symptomatic therapy was required, and the prespecified primary analysis was a linear trend test between dosage and mean change in total UPDRS score to evaluate for a trend toward efficacy. The study had 73% power to detect a 6-point change in UPDRS score; the trial was not designed to determine efficacy. Additional evaluations included comparison of the change in UPDRS for each dosage of CoQ_{10} to placebo as well assessment of the time to the need for initiation of symptomatic therapy.

CoQ_{10} was well tolerated throughout the study; there was no difference in adverse events across treatment arms, and no laboratory abnormalities were reported. The change in total UPDRS from baseline to the final visit was +11.99, +8.81, +10.82, and +6.69 for placebo, 300 mg, 600 mg, and 1200 mg CoQ_{10} respectively, showing a significant trend between dosage and mean change in UPDRS. When comparing the mean change in UPDRS for each dosage group to placebo, the 1200 mg dosage resulted in a significantly lower change than that observed

in the placebo group. After 1-month, there was no effect of CoQ_{10} treatment on the total UPDRS score as compared with baseline, suggesting that the observed changes are likely due to a disease modifying rather than a symptomatic effect. CoQ_{10} did not delay the need for initiating symptomatic therapy at any dosage evaluated. When comparing plasma CoQ_{10} levels from baseline to the end of study, there was a significant increase in all groups receiving CoQ_{10}. The mean CoQ_{10} level in subjects receiving 1200 mg CoQ_{10} was significantly greater than levels associated with 300 or 600 mg. There was no difference in CoQ_{10} levels between the 300 and 600 mg dosage arms. Activity of the electron transport chain was significantly increased by CoQ_{10} at 1200 mg daily.

A small study evaluated the effect of 360 mg of CoQ_{10} daily for 4 weeks on motor function and color vision in subjects with PD on symptomatic therapy (Muller et al., 2003). Twenty-eight subjects were randomly assigned to receive either CoQ_{10} or placebo in a 1:1 ratio and were evaluated by UPDRS at baseline and 4 weeks after treatment. No adverse events were noted. There was a significant decrease in total UPDRS scores between baseline and end of study in the CoQ_{10} group from 23.29 to 21.00. However, the total UPDRS score did not differ between groups, and there was no change in the UPDRS motor scores. Of note, subjects were not matched on baseline UPDRS scores.

The possibility of a symptomatic effect of CoQ_{10} was also raised by an open-label study in which 12 PD subjects received escalating dosages of CoQ_{10} beginning at 500 mg twice daily for 3 months followed by 500 mg three times daily for 3 months (Horstink and van Engelen, 2003). Eight subjects were receiving symptomatic therapy, and 4 were not. Evaluations were conducted at baseline, 3 months, and 6 months and included measures of walking, writing, pegboard test performances, tapping tasks, rapid alternating movements, and strength as well as the activities of daily living portion of the UPDRS. Two patients did not complete the study due to gastrointestinal symptoms. Data were analyzed using a z sum score, and a significant effect of CoQ_{10} on motor performance was reported. Yet, the authors indicate the clinical meaningfulness of the observed improvement on the tasks evaluated is not clear. No improvement on the UPDRS was noted. As this study was open label and lacked a placebo group, the results should be viewed cautiously.

The pilot data from the QE2 study (Shults et al., 2002) were encouraging and suggested that an efficacy trial of CoQ_{10} on PD progression was reasonable. However, the maximal tolerated dosage had not been identified, and a pilot study evaluating higher dosages of CoQ_{10} therefore was undertaken (Shults et al., 2004). Seventeen subjects with PD were enrolled in an open label, dosage escalation study to evaluate the safety and tolerability of CoQ_{10} at 1200, 1800, 2400, and 3000 mg. All subjects also received 1200 IU vitamin E. Subjects were seen at 2-week intervals, and the dosage was increased after at least 10 days on the prior dosage. Evaluations included assessment of adverse events, laboratory evaluations, plasma CoQ_{10} levels, and UPDRS. Four of the 17 subjects did not reach the maximum target dosage of 3000 mg due to adverse events which included orthostasis, dyspepsia, concern regarding cognitive status, and palpitations. Only dyspepsia was viewed to be related to CoQ_{10}. Other adverse events included chest pain, which was not thought secondary to study drug, and hypocalcemia of uncertain relationship. During the course of the study, there was no significant change in the motor UPDRS. Plasma CoQ_{10} levels were evaluated at each dosage, and levels were found to plateau at 2400 mg. Thus, the selection of 2400 mg for future studies was considered reasonable.

A phase II futility trial of CoQ_{10} at a dosage of 2400 mg daily with 1200 IU vitamin E has recently been completed (The NINDS NET-PD Investigators, 2007). Seventy-one subjects were enrolled and followed for one-year. The pre-specified primary outcome measure was the change in total UPDRS score from baseline to 12 months or to the visit at which symptomatic therapy was required, whichever came first. The mean change in total UPDRS was compared to a pre-specified futility threshold defined as a 30% reduction in the historically derived change in total UPDRS based on DATATOP, or a threshold of 7.46 points. Based on the pre-specified analysis, CoQ_{10} was not found to be futile. However, unplanned exploratory analyses conducted after completion of the trial using more recent placebo data suggest a futility threshold of a smaller change in UPDRS may be appropriate.

Based on the results of the QE2 study, a phase III efficacy trial of CoQ_{10} is expected to begin soon (Flint Beal, personal communication). In this study, 600 PD subjects not yet requiring symptomatic therapy will be randomized to receive CoQ_{10} at a dosage of 1200 or 2400 mg, or placebo. Subjects receiving CoQ_{10} will also receive 1200 IU vitamin E daily. The study duration is 16-month. Evaluations will be conducted at baseline and 1, 4, 8, 12, and 16 months and will include the UPDRS, Schwab and England Scale, and a measure of quality of life. The primary outcome measure will be a comparison for each treatment group to placebo of the change in total UPDRS from baseline to 16 months or to the need for symptomatic therapy, whichever comes first. Secondary outcome measures will include the change from baseline in Schwab and England Scale and in quality of life. Additionally, mean plasma levels of CoQ_{10} will be evaluated throughout the study and correlated to changes in UPDRS.

5. Trials in other neurodegenerative diseases

In addition to the aforementioned trials in ALS, HD, and PD, CoQ_{10} currently is being evaluated in both Alzheimer's disease (AD) and progressive supranuclear palsy (PSP).

Similar to the disorders discussed above, there is evidence of oxidative stress and impaired energy metabolism

in AD (Beal, 2004). A clinical trial is currently underway evaluating the safety and tolerability of CoQ_{10} and the combination of vitamin E, vitamin C, and alpha-lipoic acid in subjects with mild to moderate AD (NCT00117403). Seventy-five subjects will be randomized in a 1:1:1 manner to receive either CoQ_{10} 2400 mg daily; vitamin E 2400 IU, vitamin C 600 mg, and alpha-lipoic acid 1800 mg; or placebo for 4 months. Safety and tolerability will be evaluated, and CSF biomarkers associated with oxidative damage as well as plasma and CSF concentrations of Aβ-40 and Aβ-42 will be measured.

PSP is a rare neurodegenerative disorder characterized by postural instability, parkinsonism, supranuclear gaze palsy, and cognitive impairment. Impaired mitochondrial function may be important in the pathogenesis of this disorder (Albers and Beal, 2002). Presently there are two trials evaluating CoQ_{10} in PSP. One study is evaluating the safety and tolerability of CoQ_{10} at 5 mg/kg for a 6-week period in 30 subjects in a randomized, double-blind, placebo-controlled trial (NCT00328874). In addition, brain MRS will be conducted, and various motor and cognitive tests and assessments of ADLs will be completed in order to evaluate for evidence of an effect on disease progression. The second ongoing study in PSP is a randomized, double blind, placebo-controlled study which is anticipated to enroll 60 subjects (NCT00382824). The primary outcome measure is the rate of functional decline, and safety and quality of life are the secondary outcome measures.

6. Clinical trial considerations

There are several ongoing trials across the neurodegenerative diseases to assess the potential therapeutic role of CoQ_{10} in disease modification. To date, CoQ_{10} appears safe and well tolerated at the dosages evaluated (Medical Letter, 2006). Yet, there remain several considerations for trials of neuroprotective agents in these diseases.

While animal data using CoQ_{10} in various models of neurodegeneration have been encouraging (Beal et al., 1994,1998; Matthews et al., 1998; Ferrante et al., 2002), it is important to note that to date efficacy in an animal model has not predicted efficacy in the human disease. It is not clear whether such difficulties translating preclinical findings into effective therapeutics stems from inherent weaknesses in the animal models or from trial design issues: was the correct dosage used in the clinical trial? Did sufficient amounts of the drug reach the appropriate target in the central nervous system? Was the agent started early enough in the course of the disease? Were subjects followed for a sufficiently long period of time to detect a clinical change in what may be a slowly progressive disease? Given the rate of clinical decline which typically characterizes neurodegenerative diseases, longer-term follow-up may be needed to identify clinically meaningful benefits. As mentioned previously, there was the suggestion of an emerging difference between the CoQ and the placebo arms at about 11 months, supporting the potential importance of longer

follow-up periods. The planned study of CoQ_{10} in HD will follow subjects for 5 years. Data from pilot trials, including the difference between treatment group means and the associated variance, inform sample size and power calculations for larger phase III trials.

When viewing the CoQ_{10} trials, several dosage finding studies have been undertaken and plasma levels appear to plateau between 2400 and 3000 mg (Shults et al., 2004; Ferrante et al., 2005). The Phase III studies thus are employing maximum dosages around 2400 mg daily. Although the maximum dosage is similar across trials, the optimal formulation of CoQ_{10} is not clear. The PD trials are evaluating a formulation containing vitamin E whereas the formulation in the current ALS and HD trials does not. A bioequivalency study was conducted in which 25 healthy subjects were administered single 600 mg doses of four formulations of CoQ_{10}, and plasma CoQ_{10} concentrations were measured at various time points after drug administration (Hyson and Huntington Study Group, 2005). The inclusion of vitamin E in the formulation did not alter the bioavailability. The rationale for the inclusion of vitamin E is a synergistic effect with CoQ_{10} (Shults et al., 2004).

While the data from pilot studies are encouraging (Huntington Study Group, 2001; Shults et al., 2002), it is important to note that none of these studies has demonstrated efficacy, and the planned Phase III studies are necessary before it can be determined if CoQ_{10} is efficacious. This caution must be underscored especially since CoQ_{10} is available over-the-counter. Off-label use may expose patients to unnecessary risks as well as significant expense and highlights the need for appropriate clinical trials to be conducted.

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Review

Coenzyme Q$_{10}$ in cardiovascular disease

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Abstract

In this review we summarise the current state of knowledge of the therapeutic efficacy and mechanisms of action of CoQ$_{10}$ in cardiovascular disease. Our conclusions are: 1. There is promising evidence of a beneficial effect of CoQ$_{10}$ when given alone or in addition to standard therapies in hypertension and in heart failure, but less extensive evidence in ischemic heart disease. 2. Large scale multi-centre prospective randomised trials are indicated in all these areas but there are difficulties in funding such trials. 3. Presently, due to the notable absence of clinically significant side effects and likely therapeutic benefit, CoQ$_{10}$ can be considered a safe adjunct to standard therapies in cardiovascular disease.
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1. Introduction

Coenzyme Q$_{10}$ (CoQ$_{10}$) was discovered by Fredrick Crane in 1957. In the early 1980's Karl Folkers recognised the therapeutic potential of CoQ$_{10}$ in cardiovascular disease and used it to treat patients with heart failure (Folkers et al., 1985; Langsjoen et al., 1985). The initial results were encouraging so that further trials of CoQ$_{10}$ in ischemic heart disease and hypertension followed (Folkers et al., 1981). CoQ$_{10}$ was also used as a preoperative preparation before cardiac surgery (Judy et al., 1993) and heart transplantation (Berman et al., 2004). In subsequent years, numerous small trials of CoQ$_{10}$ in cardiovascular disease have reported promising beneficial results but others have reported no apparent effect. However few of these trials have enrolled sufficient numbers of patients to show signif-

icant differences in the major cardiac events (MACE) such as death and myocardial infarction. As a result the CoQ$_{10}$ literature is replete with multiple small trials showing promising results in terms of relief of symptoms and improvements in cardiac functional and metabolic parameters but underpowered to show improvements in MACE. Several useful meta-analyses have been published and are presented in detail in this review. These analyses have generally indicated a beneficial effect of CoQ$_{10}$ in heart failure and in hypertension.

Heart failure research is growing more demanding by the year due to the potent effects of combined therapy including ACE inhibitors, aldosterone antagonists, and beta-blockers. This therapy has decreased the therapeutic window available for adjunct therapies. As CoQ$_{10}$ is not considered a pharmaceutical drug covered by government or health insurance rebate items in the USA and most European countries, patients who use CoQ$_{10}$ therapy are involved in considerable personal cost. However this is slowly changing with Japan and some European countries (Hungary, Italy, Norway and Denmark) now granting

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licensed prescription of CoQ_{10} for heart failure and ischaemic heart disease. Despite all these obstacles and disincentives there is a large and ever growing demand by the general public for CoQ_{10} to treat cardiovascular and other diseases, either with or without medical prescription. Most major therapeutic agents have side effects that reduce the quality of life in many patients and decrease compliance with therapy. By contrast CoQ_{10} is remarkable for its absence of side effects, which is one of the reasons for patient use of it. Unlike pharmaceutical agents CoQ_{10} has very little industry financial support to drive a crucial increase in research into its therapeutic efficacy, so progress has been slow and limited. The aim of this paper is to summarise the current state of knowledge of the therapeutic efficacy and mechanisms of action of CoQ_{10} in the major cardiovascular diseases.

2. Effects of coenzyme Q_{10} in ischemic heart disease

Oxidative stress arising from the imbalance between augmented free radical production and inadequate antioxidant defence has been implicated in ischaemia-reperfusion injury. In cardiomyocytes mitochondria are abundant, (as much as 60% of cell volume), and in order to fulfil the cell's predominant dependence on mitochondrial ATP production to support contractile function and cardiac output, mitochondria are densely localised in linear formation adjacent to the myofilament contractile apparatus. In addition, mitochondria are the major source of superoxide in cardiomyocytes, particularly in response to reduced oxygen availability (Hool et al., 2005). This has lead to the development of treatments against ischaemia-reperfusion injury using agents such as CoQ_{10}, which serves multiple molecular roles in the mitochondria and beyond (Ebadi, 2001).

Improved preservation of mitochondrial ATP-generating capacity after ischaemia and reperfusion has been reported for rabbit hearts pre-treated with CoQ_{10}. These results correspond to the improved post-ischaemic preservation of myocardial contractile function and reduced creatine phosphokinase release in CoQ_{10} pre-treated hearts (Nayler, 1980). Hano et al. (1994) showed that CoQ_{10} pre-treatment improved post-ischemic recovery of high-energy phosphates and contractile function in isolated rat hearts, while preventing calcium overload and preserving diastolic function. A more recent study, also using an isolated rat heart model, demonstrated that CoQ_{10} pre-treatment improved diastolic function during reperfusion, maintained higher ATP levels, preserved coronary vasodilatation by sodium nitroprusside and increased coronary flow (Whitman et al., 1997). In a study in pigs, feeding with CoQ_{10} for 30 days rendered their hearts more resistant than controls to a combination of regional and global myocardial ischemia. Treated animals demonstrated improved contractile function accompanied by reduced release of creatine kinase and malondialdehyde in the cardiac effluent (Maulik et al., 2000).

We demonstrated the cardioprotective action of CoQ_{10} in aged ($\geqslant 70$ years) and younger (<70 years) human atrial trabeculae discarded at cardiac surgery (Rosenfeldt et al., 2002). After 30 min of immersion in either CoQ_{10} (400 μM) or vehicle solution, trabeculae were subjected to 30 min of simulated ischemia. In younger trabeculae CoQ_{10} produced a small but non-significant increase in post-ischemic contractile recovery (63.4 ± 3.4% to 71.5 ± 3.3%). However in aged trabeculae CoQ_{10} treatment produced a large increase in recovery from (53.0 ± 2.9%) to a similar level to that seen in the younger trabeculae (74.6 ± 3.5%, $P < 0.05$). That is CoQ_{10} exhibited an age-specific cardioprotective effect. CoQ_{10} is crucial for preservation of oxidative phosphorylation in the myocardium during conditions of metabolic stress with resultant reduction of myocardial damage and improvement of post-stress contractile function.

CoQ_{10} also specifically binds to a site in the inner mitochondrial membrane that inhibits the mitochondrial permeability transition pore (MPTP) (Fontaine et al., 1998; Walter et al., 2000; Papucci et al., 2003). The MPTP is a large conductance channel, which when opened can trigger the collapse of mitochondrial proton-motive force and membrane potential. In cell death signalling pathways this leads to the disruption of ionic homeostasis and oxidative phosphorylation particularly after ischemia and reperfusion (Di Lisa et al., 2003). CoQ_{10} protects creatine kinase and other key proteins from oxidative inactivation during reperfusion, a function crucial in preserving energy metabolism and cardiac performance (Crestanello et al., 1996; Chello et al., 1994; Chen et al., 1994; Taggart et al., 1996; Zhou et al., 1999; Choksi et al., 2004).

It has been reported that dietary consumption of oxidised CoQ_{10} (ubiquinone) in humans and rats leads to a marked rise in plasma of the reduced form of CoQ_{10}, ubiquinol (Mohr et al., 1992, 1999; Kaikkonen et al., 2002). Although the specific mechanism(s) of this conversion of CoQ_{10} to reduced form upon intestinal absorption are not fully elucidated, the mucosal GSH peroxidase/oxidized glutathione (GSSG) reductase system which catalyses the reduction of lipid hydroperoxides has been implicated (Aw et al., 1992). CoQ_{10} is carried mainly by lipoproteins in the circulation, predominantly in its reduced form, ubiquinol. Ubiquinol acts as an antioxidant in plasma lipoproteins, lowering the oxidation rate of dietary fatty acids transported in the lipoproteins (Alleva et al., 1995; Tomono et al., 1986; Frei et al., 1990; Stocker et al., 1991). During its anti-oxidative action ubiquinol is oxidised to ubiquinone.

CoQ_{10} has an important role in preventing the initiation and/or propagation of lipid peroxidation in plasma lipoproteins and membrane proteins. Ferrara et al. (1995) demonstrated that chronic treatment with CoQ_{10} in rats protected against cardiac injury due to oxidative stress created by hydrogen peroxide (H_2O_2) in the heart. CoQ_{10} can inhibit lipid peroxidation in mitochondria (Glinn et al., 1997), protein oxidation (Ernst et al., 2004) and DNA

oxidation (Tomasetti et al., 1999). After its antioxidative action, ubiquinone can be recycled to the antioxidant, active, reduced ubiquinol form via the mitochondrial Q cycle. CoQ_{10} importantly is also responsible for transforming Vitamin E radicals to regenerate the reduced (active) α-tocopherol form of Vitamin E (Constantinescu et al., 1994).

As well as playing a crucial role in the mitochondrial respiratory chain for ATP production, CoQ_{10} also plays a crucial role in extra-mitochondrial electron transfer such as in the regulation of NADH oxido-reductase activity in the plasma membrane (Lawen et al., 1994; Villalba et al., 1997), and also has potential redox activity in both Golgi apparatus and lysosomes (Crane et al., 1984).

There is another potential molecular action of CoQ_{10} that to date has had little investigation. This relates to the capacity of CoQ_{10} to regulate and alter genomic expression. CoQ_{10} has been shown to target the expression of multiple genes, particularly those involved in cell signalling and intermediary metabolism (Groneberg et al., 2005). Linnane and colleagues (2002), have reported that 25–30 days oral intake of CoQ_{10} (300 mg/day) by patients waiting for hip surgery resulted in a significant change in the expression of 115 genes, 47 up-regulated and 68 down-regulated, as measured by differential gene array chip methodology in vastus lateralis muscle biopsy. Many of these genes included those governing nuclear and other enzymes, transcription factors, muscle fibre components, growth factors, receptors and receptor-activated signal transduction and other metabolic pathways. This work thus opens an entire new avenue of investigation to tease out other specific mechanisms of CoQ_{10}'s pleiotropic actions. Thus gene regulation and control of metabolic flux may explain many of the cardiovascular and other actions of CoQ_{10} whereby it may act in a beneficial way at multiple sites in the pathophysiological cascade.

At a clinical level, protection against myocardial ischemia has been demonstrated in two double blind placebo-controlled crossover trials of CoQ_{10} in patients with ischaemic heart disease by a reduction in angina, improved exercise tolerance and a reduction in ischaemic changes on ECG (Kamikawa et al., 1985; Schardt et al., 1985). These beneficial effects may be related to increased efficiency in myocardial mitochondrial energy production as we have demonstrated in patients with ischaemic heart disease following the treatment with CoQ_{10} (Rosenfeldt et al., 2005).

3. Coenzyme Q_{10} in heart failure

3.1. Loss of efficient respiratory chain function, augmented ROS and diminished ATP

Central to the loss of contractile function in heart failure is the inability of mitochondria to adequately supply the myocardium with ATP, resulting in energy deprivation in the cell and potentially necrotic/apoptotic cell death. It is estimated that the majority of mitochondrial ATP-derived energy supports myocardial contraction; and the maintenance of ion homeostasis; these activities account, respectively for 75% and 25% of cardiomyocyte energy consumption (Giordano, 2005). However, the underlying molecular causal events leading to metabolic dysfunction are poorly understood. The production of reactive oxygen species (ROS) has been shown to increase in the failing heart, and, mitochondrial proteins and lipids may be targets of oxidative damage in the failing heart due to their close proximity to sites of superoxide production. (Fig. 1).

Reduced ATP synthesis, as measured by a lowering of state III (ADP-coupled) respiration has been demonstrated in isolated cardiac mitochondria from failing animal (Sharov et al., 1998) and human (Sharov et al., 2000) hearts, relative to non-failing controls. In an attempt to identify possible causes for this decline, many studies have focused on the measurement of the electron transport chain enzymes in a number of human cardiomyopathies. Most notably, complex I (Scheubel et al., 2002), complex III (Jarreta et al., 2000), and complex IV (Arbustini et al., 1998), have been identified as dysfunctional in end-stage human heart failure. A direct causal relationship, however, has proven elusive over the years, with a poor correlation between reduced complex activities and the severity of disease (Arbustini et al., 1998). This may be partly explained by a large reserve capacity for activity of each respiratory protein complex. For example, up to 50% inhibition of complex I, or IV activity, is required before a significant decline in state III respiration is apparent (Lucas and Szweda, 1999).

In contrast, Krebs cycle enzymes involved in the regulation of substrate metabolism appear to exert a more direct control over energy output, with a close correlation between the loss of α-ketoglutarate dehydrogenase (KGDH) activity and decreased state III respiration (Humphries et al., 1998). Due to their close interaction with Complex I, a major site of mitochondrial superoxide formation, NADH-linked enzymes such as KGDH and isocitrate dehydrogenase (ICDH) may be susceptible to ROS-dependent perturbation. Reduced ICDH activity (∼30%) has been demonstrated as an early marker of hypertrophy before the onset of ventricular dysfunction, in transgenic hypertrophic cardiomyopathic mice (Lucas et al., 2003) and spontaneously hypertensive rats (SHR), (Benderdour et al., 2004). The decline in α-KGDH, pyruvate dehydrogenase (PDH) and ICDH activity coincides with the formation of protein thiol adducts with 4-hydroxy-2-nonenal (HNE), a lipid peroxidation aldehyde formed via reaction between arachidonic acid and superoxide (Esterbauer et al., 1991; Humphries and Szweda, 1998; Humphries et al., 1998; Benderdour et al., 2003). Reduced α-KGDH activity together with increased HNE-adduct formation has also been described in aged rats (Humphries and Szweda, 1998; Lucas and Szweda, 1999). However, the specific role that perturbation of these enzymes play in contributing to an energy deficit in the failing heart awaits detailed examination.
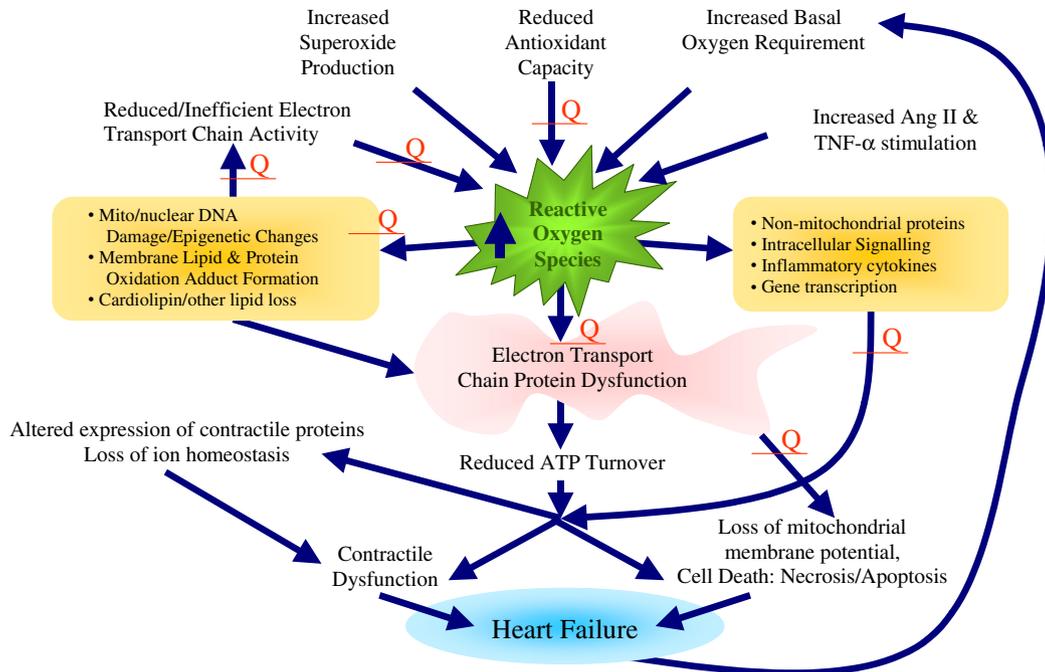
Fig. 1. CoQ$_{10}$ treatment (as indicated by "Q") may intervene in the scheme where augmented ROS production contributes to post-ischemic injury and progression to heart failure.

Nevertheless it is apparent that diminished mitochondrial energy metabolism in the failing heart involves dysfunction in Krebs cycle regulation, NADH supply and activity of electron transport chain proteins. Thus CoQ$_{10}$ may act in a beneficial way at multiple sites in the pathophysiological cascade of advancing heart failure (Fig. 1).

Increased myocardial levels of oxidative stress markers have been demonstrated in animal models of heart failure produced by coronary ligation (Hill and Singal, 1996), pressure overload (Dhalla and Singal, 1994) and rapid cardiac pacing (Ide et al., 2000). ROS are key pathophysiological mediators in myocardial remodelling in heart failure (Singal et al., 1993). In human heart failure, there is also evidence of increased levels of oxidative stress markers such as malondialdehyde (MDA) in serum (Belch et al., 1991), and isoprostanes in urine (Cracowski et al., 2000). Furthermore the levels of these markers correlate with the severity of heart failure.

Elevated MDA concentration has been measured in the plasma of patients with symptoms of moderate congestive heart failure (NYHA III, left ventricular ejection fraction less than 40%), compared to age-matched non-failing controls (ejection fraction greater than 40%), which increased with the duration (years) of congestive heart failure (Diaz-Velez et al., 1996). A significantly high concentration of plasma MDA and reduced thiol has also been reported in heart failure patients with underlying coronary artery disease (McMurray et al., 1990). Mak and co-workers, (2000) demonstrated that total aldehydes are elevated in the plasma of heart failure patients, with a strong negative correlation between total aldehydes and contractility ($+dP/dt$) as well as with increased time to relaxation

($-dP/dt$). Keith et al. (1998) also reported a correlation between severity of heart failure and elevated lipid peroxides and MDA in both ischemic heart disease and dilated cardiomyopathy patients with end-stage heart failure. Another indirect marker of oxidative stress, 8-iso-prostaglandin F_{2a}, derived from the oxidation of arachidonic acid, is increased in the pericardial fluid of patients in proportion to the severity of heart failure of ischemic and/or valvular disease origin (Mallat et al., 1998).

Direct evidence of augmented myocardial superoxide production in the failing heart has been established in studies using electroparamagnetic spin resonance (EPR) spectroscopy ("spin-trapping"). Increased superoxide production has been demonstrated in the myocardium of explanted human heart failure tissue compared to non-failing donor heart muscle (Sam et al., 2005), confirming the results obtained in a pacing-induced animal model of heart failure (Ide et al., 1999). However, *in vivo*, real time demonstration of increased cardiac superoxide metabolism in human heart failure is yet to be reported.

A significant contribution of oxygen radical generation in the cardiomyocyte originates within mitochondria during oxidative phosphorylation. It is estimated that during normal metabolism 1–2% of oxygen is incompletely converted to H_2O during electron transfer, resulting in the formation of ROS such as the superoxide ($^{.}O_2-$) and hydroxyl (OH^-) anions and H_2O_2 (Turrens, 1997). The reactivity of oxygen derives from its incomplete pairing of electrons in the outer electron shell, and thus single electron transfer forms the superoxide anion, $^{.}O_2-$ (Giordano, 2005). The proposed sites of mitochondrial superoxide formation are at Complex I (NADH dehydrogenase) (Turrens and

Boveris, 1980), and the ubisemiquinone site of Complex III (Turrens et al., 1985), as demonstrated using targeted inhibitors. Due to the location of sites of oxygen radical formation, complex I is expected to release superoxide towards the mitochondrial matrix, whereas superoxide generated at complex III may be directed towards both the matrix and intermembrane space (Turrens et al., 1985; Lesnefsky et al., 2001). Through the use of mass spectroscopy, protein subunits protruding into the mitochondrial matrix have been shown to be most at risk from oxidative damage as targets of HNE binding, or carbonyl and nitrosyl modification under basal conditions. Increased superoxide formation by Complex I has been directly measured in the sub-mitochondrial fraction from failing cardiomyocytes, together with a significant loss of complex I activity (Ide et al., 1999), implicating the mitochondrial electron transport chain as a significant contributor to the pathogenesis of heart failure.

It has been proposed that the amount of superoxide formed is increased under state IV as opposed to state III respiration, due to lower ADP availability and increased molecular oxygen, whereas uncoupling conditions lower the production of ROS. Basal respiration has been shown to increase in patients with congestive heart failure (Poehlman et al., 1994) by increased resting oxygen consumption, indicating an underlying increase in basal ROS production in the diseased state. Under conditions of inefficient mitochondrial respiration due to reduced complex I activity, ROS production may also be increased (Pitkanen and Robinson, 1996; Luo et al., 1997).

3.2. Antioxidant therapy

Li et al. (1995), demonstrated, in a superoxide dismutase 'knock-out' genetic mutant mouse model, a rapid onset of severe cardiomyopathy causing death in neonates that implicated inadequate endogenous regulation of superoxide metabolism. Experimental animal studies have reported beneficial effects of antioxidant therapy during the development of heart failure. Vitamin E in guinea pigs with pressure overload can prevent the transition from compensated hypertrophy to heart failure (Dhalla et al., 1996). Similarly probucol, a lipid-lowering agent with antioxidant actions, can protect against heart failure induced by adriamycin (Singal et al., 1995) and diabetes (Kaul et al., 1995) – see Kukin and Fuster (2003) for a detailed review. There are multiple molecular, cellular and neurohumoral mechanisms that contribute to the syndrome of heart failure and it is likely that oxidative stress is involved in some or all of these processes. There is no doubt that antioxidant therapy can attenuate oxidative stress. However, many of the early clinical trials of antioxidant therapy for heart failure were negative. This may be explained by the use of ineffective, incorrectly dosed agents (Cohn, 2003). However the results of using more potent antioxidants such as CoQ_{10} that have other beneficial actions have been more encouraging (Langsjoen et al., 1985; Poggesi

et al., 1991; Permanetter et al., 1992; ?; Munkholm et al., 1999; Watson et al., 1999). A recent small ($n = 23$) trial in patients with NYHA Class II and III heart failure using a cross-over design, showed that four weeks of CoQ_{10} (100 mg tid) therapy improved exercise capacity (VO_2), cardiac ejection fraction and endothelium-dependent brachial artery dilation (Belardinelli et al., 2006). Similar effects were achieved by physical exercise training and these effects were in general additive to those of CoQ_{10} therapy. However most trials have been open label and in some, only 50% of patients took angiotensin converting enzyme (ACE) inhibitors that are now standard therapy for heart failure. Differences in etiology, the stage of heart failure progression and therapeutic treatment history complicate any comparisons between studies and patient groups. Although CoQ_{10} treatment for heart failure has been claimed to ameliorate symptoms, improve quality of life and reduce rates of hospitalisation, various limitations of such studies and the reported lack of beneficial effects by others (Khatta et al., 2000), indicate the need for large multi-centre, closely monitored double-blind placebo controlled trials in heart failure.

3.3. Meta-analyses of randomised trials of coenzyme Q_{10} in heart failure

A meta-analysis showing a beneficial effect of CoQ_{10} in heart failure was published in 1997 (Soja and Mortensen, 1997). We updated their findings by performing a meta-analysis of randomised trials of CoQ_{10} in heart failure published up to 2003 (Rosenfeldt et al., 2003). Only prospective, randomised, double-blinded and placebo-controlled trials were included in that analysis. The only three parameters with adequate numbers of subjects for meaningful analysis were CoQ_{10} levels (five trials), ejection fraction at rest (seven trials) and mortality (five trials). Other parameters were measured in only two trials each. For CoQ_{10} levels (279 patients) the weighted mean difference was 1.4 µg/mL, representing an increase of 161%. For ejection fraction at rest (384 patients) the weighted mean difference showed a trend in favour of CoQ_{10}, of 1.9% (95% confidence limits −0.13 to 3.9%).

An updated meta-analysis has been recently published (Sander et al., 2006). This meta-analysis included eleven randomised trials of coenzyme Q_{10} in heart failure, including both cross-over and parallel trial designs (Table 1). The main endpoint of resting ejection fraction showed a 3.7% net improvement (1.59–5.77; $P < 0.0006$; Fig. 2). Stroke index also increased by 5.8 ml ($P = 0.02$). Subgroup analyses showed that the ejection fraction improvement was more pronounced when studies of NYHA class IV were excluded, when only idiopathic cardiomyopathy was evaluated and among patients not receiving angiotensin converting enzyme (ACE) inhibitors. When the results were re-analysed using a less conservative statistical test (fixed effects modelling) than was used in the initial analysis

Table 1
Prospective randomised clinical trials of CoQ$_{10}$ for heart failure

Trial	Age	Dose used	Treatment duration	Etiology of HF	NYHA class	Other HF medications
Crossover trials						
Hoffman-Bang et al., 1995 (n = 69)[27]	61 (10)	100 mg QD	3 mo (no washout)	Ischemic and nonischemic (idiopathic, hypertensive, valvular, and other)	II–IV (76% Class II)	75% digoxin, 96% diuretics 60% ACE inhibitor, no BB
Langsjoen et al., 1985 (n = 19)[28]	63	33.3 mg T1D	3 mo (no washout)	Idiopathic	III–IV	100% digoxin, 94% diuretics-No ACE inhibitor or BB
Morisco, 1994 (n = 6[29])	50 (6.7)	50 mg TlD	1 mo (no washout)	4 CAD and 2 idiopathic	II–IV	Nitro derivatives No ACE inhibitor or BB
Poggesi et al., 1991 (n = 18)[31]	67 (2.3)	50 mg BID	2 mo	13 idiopathic, 7 ischemic (only 18 completed the study)	II–III	Digoxin, diuretics, ACE inhibitor
Serra, 1991 (n = 20)[31]	59 (6.6)	60 mg QD	1 mo (no washout)	13 CAD, 7 hypertensive	II–III	Digoxin, diuretics, nitrates
Watson et al., 1999 (n = 27)[32]	55 (11)	33 mg TID	3 mo	77% idiopathic, 23% ischemic	Mean 41 mo duration andEF <35%	80% digoxin, 93% diuretics 83% nitrates or hydralazine, 100% ACE inhibitor, no BB
Parallel trials Keogh et al., 2003 (n = 35)[33]	62 (8)	50 mg TID	3 mo	Ischemic, valvular; idiopathic	II–III EF < 40%	71% digoxin, 91% diuretics, 22% nitrates or hydralazine 100% ACE inhibitor, no BB
Khatta et al., 2000 (n = 46)[34]	64	200 mg/d	6 mo	59% ischemic	III–IV (91% Class III) EF <40%	96% diuretics, 100% digoxin, 100% ACE inhibitor or other vasodilators, 78% BB
Munkholni 1995 (n = 22)[35]	57	100 mg BID	3 mo	Ischemic or dilated	II-III EF < 45%	55% digoxin, 86% diuretics, 95% ACE inhibitor, no BB
Judy, 1993 (n = 10)[18]	66	100 mg/d	6 mo	Various etiologies	IV	Unknown
Permanetter et al., 1992 (n = 25)[19]	52	100 mg/d	3 mo	Idiopathic	I-III (60% Class III)	92% digoxin, 64% diuretics, 44% nitrates or nifedipme

From Sander et al., 2006, with permission.
ACE, angiotensin-convertins enzyme; BB, beta-blockers; BID, twice daily; EF, ejection fraction; HF, heart failure; NYHA, New York Heart Association; QD, once daily, TID, three times daily.
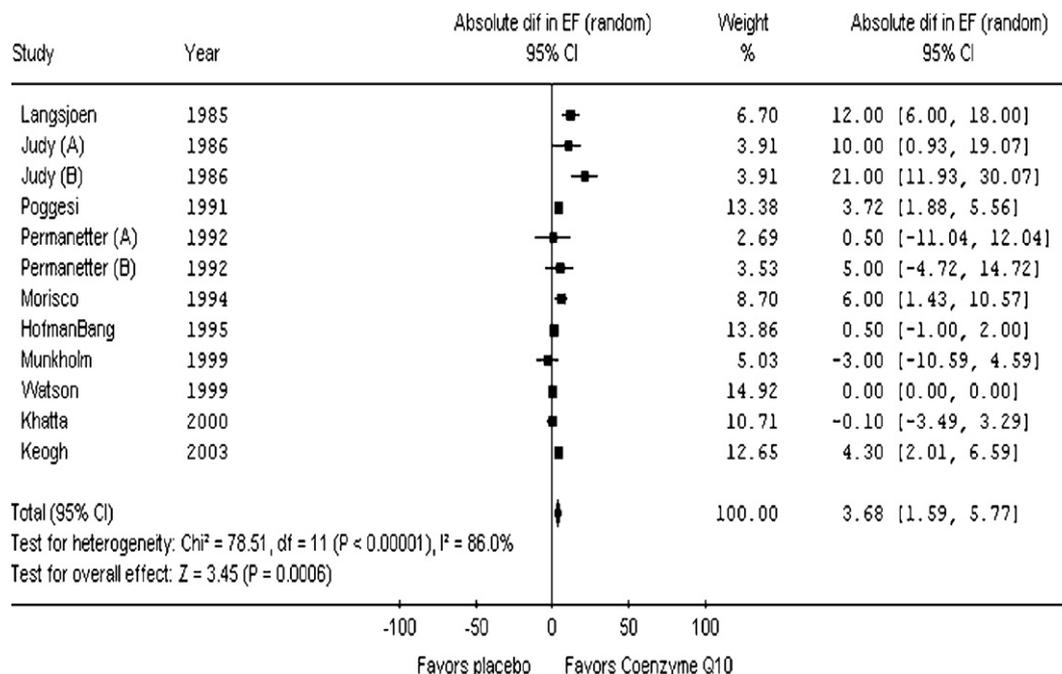

Fig. 2. Forrest plot of the effect of CoQ$_{10}$ on cardiac ejection fraction. From Sander et al., 2006, with permission.

(random effects modelling), increases in cardiac output, cardiac index and stroke volume all became significant.

Unfortunately most of the published trials to date have been underpowered to detect significant differences, particularly in major endpoints such as mortality. Trials to detect a mortality difference would need to be prohibitively large, requiring 2000 or more patients per group. For the future, a reasonable expectation would be to conduct a multinational prospective, randomised trial containing 300–400 patients per group to make a more definitive conclusion as to the effects of CoQ_{10} on cardiac function and symptoms in cardiac failure. Such a trial, the "Q-symbio" trial is currently in progress (Mortensen, 2003).

In light of the encouraging findings of the above mentioned meta-analyses, it is not unreasonable to recommend to patients with symptomatic heart failure despite conventional therapy or those who are experiencing side effects of conventional therapy (especially ACE inhibitors), to take 150–300 mg of CoQ_{10} daily and to monitor CoQ_{10} blood levels and the clinical response.

3.4. Statin sensitivity and coenzyme Q_{10} deficiency

The 3-hydroxy-3-methylglutaryl Coenzyme A (HMG CoA) reductase inhibitors or "statins" are at present one of the most widely prescribed drugs in the Western World. Although the effect of statins on CoQ_{10} levels will be covered in detail by others in this journal issue, it is important to highlight here apparent complications related to their use in cardiovascular disease. From 1990 to 2003, 15 studies in humans have been published evaluating the effects of statins on CoQ_{10} metabolism (Langsjoen and Langsjoen, 2003). Nine of these were randomised controlled trials, and eight of these demonstrated significant depletion of plasma CoQ_{10} due to statin therapy. De Pinieux et al. (1996) reported raised lactate to pyruvate ratios in statin-treated patients, in association with CoQ_{10} depletion and mitochondrial dysfunction.

Thus some statins, mainly lipid soluble types, may decrease body CoQ_{10} levels below the threshold that is required for numerous redox-dependent processes. This depletion could be particularly important in the elderly where CoQ_{10} levels are generally low. Adverse effects of statin-induced CoQ_{10} reduction have been reported at a mitochondrial level but infrequently at a clinical level. When they do occur these adverse effects may be correctible by concurrent administration of CoQ_{10}. By contrast in a large study of patients with heart failure statin use was associated with lower risks of death or hospitalisation among patients with or without coronary artery disease (Go et al., 2006). This result concurs with the findings of several cohort studies of heart failure patients treated with statins (Udell and Ray, 2006).

Beneficial effects of statins in heart failure may relate to their pleotrophic effects which include improved endothelial function and stimulation of cellular antioxidant and anti-inflammatory processes. In most heart failure patients these beneficial effects may outweigh the detrimental effects of CoQ_{10} depletion, particularly if such depletion is only partial. Further studies examining the specific mechanisms of cardiovascular-specific local synthesis of CoQ_{10} and molecular mechanisms of statin action on cellular metabolism are needed.

3.5. Prevention of anthracycline-induced cardiotoxicity

Doxorubicin and other anthracyclines are among the most potent chemotherapeutic agents for the treatment of a wide variety of tumours. However their usefulness is limited by dose-related cardiotoxicity (Tokarska-Schlattner et al., 2006) that leads to arrhythmias and myocardial depression during therapy and to cardiomyopathy that can present years after treatment (Abu-Khalaf et al., 2006). There is increasing evidence (summarised in three reviews: van Dalen et al., 2005; Conklin, 2005; Wouters et al., 2005), that CoQ_{10} administration can prevent or reduce these undesirable side effects.

The dose-related cardiomyopathy induced by anthracyclines may be explained largely by the induction of irreversible oxidative damage to cardiac mitochondria. These organelles are highly susceptible to the effects of anthracyclines due to the presence of a unique enzyme (a NADH dehydrogenase) in their inner mitochondrial membrane (Conklin, 2005). This enzyme reduces anthracyclines to their semiquinones ultimately resulting in severe oxidative damage to mitochondrial DNA leading to apoptosis of cardiomyocytes. CoQ_{10} by its antioxidant action and high concentration in the inner mitochondrial membrane can reduce this oxidative damage.

CoQ_{10} has been shown to protect against anthracycline-induced lipid peroxidation in beef heart mitochondria isolated rat hearts and intact mice, rats and rabbits (Conklin, 2005). These protective effects are not normally seen with other antioxidants such as vitamin E. Three non-randomised clinical trials have shown that the simultaneous treatment of patients with CoQ_{10} and anthracyclines decreases cardiotoxicity without reducing the anti-tumour effect. However one randomised trial showed no significant difference in effect between CoQ_{10} treated patients and controls (Wouters et al., 2005).

We conclude that the use of CoQ_{10} for the prevention and treatment of anthracycline-induced cardiotoxicity is promising on the basis of numerous animal studies and a small number of clinical trials. As with many of the other clinical applications of CoQ_{10} therapy, larger randomised clinical trials are needed to confirm or deny the preliminary findings, to determine the optimum dose of CoQ_{10} and whether CoQ_{10} treatment preserves (or even enhances) anti-tumour effects. In the meantime a case can be made for combining CoQ_{10} with anthracycline therapy and carefully monitoring the results in terms of cardiotoxicity and anti-neoplastic effects.

4. Coenzyme Q$_{10}$ in hypertension

4.1. Antihypertensive effects and clinical efficacy

Hypertension is currently managed by a variety of medications. These are effective in reducing blood pressure, but many have undesirable side effects such as renal or cardiac dysfunction, cough and mental depression. CoQ$_{10}$ has been shown in laboratory and clinical studies to have a hypotensive effect (Yamagami et al., 1975; Singh et al., 1999; Burke et al., 2001; Hodgson et al., 2002). Since 1975, many studies have described the potential of CoQ$_{10}$ to lower blood pressure in hypertensive patients. Negligible side effects have been reported even with high doses of CoQ$_{10}$. A study of subjects with type 2-diabetes showed that CoQ$_{10}$ therapy lowered blood pressure and improved glycemic control (Hodgson et al., 2002). However, despite these reports the current role of CoQ$_{10}$, if any, in the treatment of hypertension is unclear. We recently reviewed the published clinical trials of CoQ$_{10}$ in the management of hypertension in terms of its therapeutic effect and side effect profile and conducted a meta-analysis of the pooled results using STATA v.8.2 software with the Cohen method of meta-analysis for weighted mean difference with respect to continuous variables (Rosenfeldt et al., 2007). We identified 12 studies that reported a total of 362 patients in which CoQ$_{10}$ had been used in the therapy of hypertension (Table 2).

These comprised three randomized controlled clinical trials (Yamagami et al., 1986; Singh et al., 1999; Burke et al., 2001), one cross-over study (Digiesi et al., 1990) and eight open label observational studies without a control group (Yamagami et al., 1975, 1976, 1977; Folkers et al., 1981; Montaldo et al., 1991; Digiesi et al., 1992; Digiesi et al., 1994; Langsjoen et al., 1994). When trial results were pooled, CoQ$_{10}$ produced a reduction of up to 17 mmHg in systolic and 10 mmHg in diastolic blood pressure, (Rosenfeldt et al., 2007).

4.2. Mechanism of antihypertensive action

An increase in oxidative stress is well documented in hypertensive states (Koska et al., 1999). In blood vessels, oxidative stress increases the production of superoxide radicals that rapidly react with endothelial nitric oxide (NO) to form peroxynitrite, thus reducing NO availability (Grunfeld et al., 1995). This reduction impairs the ability of endothelium to induce NO-mediated relaxation of underlying smooth muscle with resultant vasoconstriction and increased blood pressure. The primary action of CoQ$_{10}$ in clinical hypertension is vasodilatation, via a direct effect on the endothelium and vascular smooth muscle resulting in decreased peripheral resistance accompanying lowered blood pressure and unchanged cardiac output (Folkers et al., 1981; Digiesi et al., 1992, 1994). In patients with diabetes or dyslipidemia CoQ$_{10}$ improves endothelial function and lowers blood pressure (Watts et al., 2002). Thus the benefit of CoQ$_{10}$ is as a potent lipid-soluble antioxidant that preserves NO availability and reduces vasoconstriction and augmented blood pressure. It should be noted however that in normal animals or humans, CoQ$_{10}$ has no direct vasodilatory or hypotensive effect. This confirms that the hypotensive effect of CoQ$_{10}$ is specific to the state of enhanced oxidative stress occurring in hypertensive patients.

4.3. Clinical implications

Being devoid of significant side effects, CoQ$_{10}$ may thus have a useful clinical role as an adjunct or alternative anti-hypertensive to conventional agents such as diuretics and ACE inhibitors in the treatment of hypertension. In support of this, in one study 50% of subjects treated with CoQ$_{10}$ were able to cease at least one of their other hypertensive medications (Langsjoen et al., 1994). As CoQ$_{10}$ has the potential in hypertensive patients to lower systolic blood pressure by up to 17 mmHg and diastolic pressure by up to 10 mmHg

Table 2
Trials of CoQ$_{10}$ in hypertension

	Studies (n)	Patients (n)	Systolic blood pressure (mmHg)			Diastolic blood pressure (mmHg)		
			Before	After	Difference	Before	After	Difference
Randomised trials								
Treatment group	3	63	167.7 (163.7–171.1)	151.1 (147.1–155.1)	−16.6 (−20.6 to −12.6)	103 (101.0–105.0)	94.8 (92.8–96.8)	−8.2 (−10.2 to −6.2)
Control group		57	166 (162.1–170.0)	163.9 (159.9–167.9)	−2.1 (−6.1 to 1.9)	102.5 (100.2–104.7)	100.5 (98.3–102.8)	−1.9 (−4.2 to 0.3)
Crossover study								
Treatment phase	1	18	167 ± 2.6	156 ± 2.3	−11	103 ± 1.2	95 ± 1.0	−8
Control phase				166 ± 2.4			103 ± 1.0	
Open label studies								
	8	214	162 (158.4–165.7)	148.6 (145.0–152.2)	−13.5 (−17.1 to −9.8)	97.1 (95.2–99.1)	86.8 (84.9–88.8)	−10.3 (−12.3 to −8.4)

From Rosenfeldt et al. (2007), with permission.

without significant side effects, there is now a convincing case for conducting a high quality prospective randomised trial of CoQ_{10} in order to validate the results of this meta-analysis. In the current era of improved management of hypertension it would be unethical to conduct a placebo-controlled trial in hypertensive patients.

The ideal trial would be one comparing CoQ_{10} with an ACE inhibitor or diuretic as in the ANBP2 trial (Wing et al., 2003) to demonstrate non-inferiority of CoQ_{10}. Two types of trials would be useful. The more conventional type would be one with the primary endpoint of death and major cardiac events such as stroke. Such a trial would need to include several thousand patients for adequate statistical power. The second type of trial would be one with end points such as adequacy of blood pressure control, improvement in cardiac function, improvement in exercise tolerance and quality of life, as well as prevalence of adverse effects. Until the results of such trials are available it would seem acceptable to add CoQ_{10} to conventional anti-hypertensive therapy, particularly in patients who are experiencing intolerable side effects of conventional anti-hypertensive therapy. CoQ_{10} may also have a particular therapeutic role in hypertensive patients with consistently increased levels of oxidative stress as in diabetes or renal failure.

5. Coenzyme Q_{10} in cardiac surgery

Coenzyme Q_{10} has been used in the cardiothoracic surgical setting in order to offset reperfusion-related increases in free radical formation and oxidative stress. From 1982 to 2004 at least eight controlled trials of CoQ_{10} in cardiac surgery have been published (Tanaka et al., 1982; Shiguma et al., 1983; Sunamori et al., 1991; Judy et al., 1993; Chello et al., 1994; Taggart et al., 1996; Zhou et al., 1999; Rosenfeldt et al., 2005). All but one of these have shown a beneficial effect of some kind. The one trial showing an absence of effect (Taggart et al., 1996) used oral CoQ_{10} for only 12 h before surgery, an inadequate time frame for sufficient dosing to increase myocardial levels. A prospective randomised placebo controlled trial from our unit of 300 mg per day of oral CoQ_{10} for two weeks preoperatively in 121 coronary bypass or valve replacement procedures showed increased mitochondrial CoQ_{10} content, increased efficiency of mitochondrial energy production and improved contractile function in myocardial trabeculae (Rosenfeldt et al., 2005).

6. Treating cardiac complications in Friedreich's ataxia with coenzyme Q_{10}

Friedreich's ataxia (FRDA) is an autosomal recessive degenerative disease (1 in 30,000 live births) characterized by loss of large sensory neurones in the dorsal root ganglia and degeneration of the dorsal columns of the spinal cord, progressive limb and gait ataxia, loss of deep tendon reflexes, loss of the sense of position and vibration in the lower limbs, dysarthria and hypertrophic cardiomyopathy.

The genetic abnormality has been mapped to chromosome 9q13 which encodes the protein frataxin. The genetic abnormality accounting for 98% of cases is the expansion of a GAA triplet repeat in intron 1 of the FRDA gene. This results in decreased frataxin mRNA levels which leads to lower levels of frataxin protein detected in muscle and brain of these patients.

The exact function of frataxin in humans is still unknown although evidence from yeast and mice indicate it has a key role in mitochondrial iron homeostasis. Endomyocardial biopsies in FRDA patients show deficient activity of the iron-sulphur (Fe–S) cluster containing proteins, namely complexes I, II and III of the mitochondrial respiratory chain. It appears that mitochondrial iron accumulation in FRDA is a consequence of deregulation of a mitochondrial iron import system triggered by the decreased amount of frataxin, normally acting as a regulator of the mitochondrial iron homeostasis. Studies of skeletal muscle from these patients have demonstrated a profound deficit of mitochondrial ATP production. Current evidence suggests that this frataxin deficiency results in impaired mitochondrial respiratory chain function due to the mechanisms outlined above. In addition, increased oxidative damage is seen in these patients and is likely to be a secondary consequence of impaired respiratory chain function and increased free radical generation related to Fenton reactions due to excess intracellular iron accumulation.

CoQ_{10} therapy in conjunction with vitamin E has been assessed in a small study of FRDA patients showing significant improvements in heart and skeletal muscle energetics after 6 months therapy (Cooper and Schapira, 2003). Idibenone, a short chain analogue of CoQ_{10}, is a potent free radical scavenger that crosses the blood brain barrier, and has been recommended in the treatment of FRDA. However a one-year study of idibenone in 29 Friedreich ataxia patients showed a reduction in left ventricular hypertrophy but no improvement in neurological condition (Mariotti et al., 2003).

7. Getting coenzyme Q_{10} into the heart-dietary and endogenous synthesis

CoQ_{10} is poorly absorbed from food in the gut: only 10% of CoQ_{10} contained in a meal is absorbed (Weber et al., 1997). Bioavailability from a standard oral dose is low, being only 2–4% (Zhang et al., 1995), however, it is improved when CoQ_{10} is in an oily suspension (Bhagavan et al., 2001). Water soluble gel formulations have been developed for improved CoQ_{10} absorption (Chopra et al., 1998a,b).

Oral supplementation of CoQ_{10} leads to an elevation of plasma levels, with peak plasma CoQ_{10} levels occurring between 5 and 10 h after ingestion (Tomono et al., 1986). CoQ_{10} is absorbed slowly from the gastrointestinal tract, probably due to its high molecular weight and low water solubility. Following absorption from the gastrointestinal

tract, CoQ_{10} is taken up by chylomicrons and transported to the liver for packaging into very low density lipoproteins (VLDL). From there it is transported to various tissues according to their requirement. Orally administered CoQ_{10} appears to have a low clearance rate from the plasma, and therefore has a relatively long plasma half-life of 34 ± 5 h, with excretion predominantly through the biliary tract. Approximately 90 per cent of the steady state serum concentration can be achieved after 4 days of dosing.

Analysis of the distribution of CoQ_{10} in the circulation shows that about 60% of CoQ_{10} is transported by LDL, and less than 30% by HDL (Alleva et al., 1995). However, in the absence of significant exogenous supply of CoQ_{10}, individual tissues must rely on their own production as endogenously produced CoQ_{10} is not transported within the body or redistributed to any great degree. Although CoQ_{10} is present in a normal diet, with red meat and poultry being the richest sources, endogenous production appears to be the main source in humans (Weber et al., 1994). Whether it is in the diet or pharmaceutical supplementation, it is the oxidised form of CoQ_{10} that is ingested and absorbed. The CoQ_{10} is then reduced in the circulation, most likely in the red blood cells (Stocker and Suarna, 1993). Thus most CoQ_{10} in the blood is present as the reduced form ubiquinol, consistent with its activity as an antioxidant in the circulation.

In humans, the fate of exogenously administered CoQ_{10} once it reaches the circulation has not been completely elucidated. Work in rat hearts has shown that exogenously administered labelled CoQ_{10} is incorporated into subcellular organelles, especially in the inner membranes and matrix of mitochondria, within 72 h of administration (Nakamura et al., 1980). It has also been demonstrated that incubation of beef heart submitochondrial particles in a CoQ_{10} solution leads to incorporation of CoQ_{10} in their membranes (Lenaz et al., 1994). The same authors found that kinetic saturation with CoQ_{10} could not be achieved because of the intrinsic insolubility of the molecule, thus concluding that the upper limit of electron transfer from NADH is a function of CoQ_{10} solubility in the membrane phospholipids. The bioavailability of different CoQ_{10} preparations varies markedly, with a hydrosoluble gel being superior to oil based preparations, with dry powder and tableted preparations being the least bioavailable (Molyneux et al., 2004).

Our own investigations into the use of CoQ_{10} in cardiac surgery patients receiving 300 mg/day orally of CoQ_{10} dispensed in soy bean oil demonstrated a four-fold increase in serum concentration of CoQ_{10}, and a 2.5-fold increase in of CoQ_{10} in atrial myocardium which included a 2.4-fold increase of CoQ_{10} in atrial mitochondria (Rosenfeldt et al., 2005).

It has recently been reported that three important genes are crucial to synthesis of CoQ_{10}. The CoQ_3 gene permits production of enzymes that catalyse two *O*-methylations whereas the CoQ_7 gene is crucial for processes leading to hydroxylation of the benzoquinone ring (Vajo et al., 1999;

Jonassen and Clarke, 2000). The CoQ_2 gene encodes for *p*-hydroxybenzoate:polyprenyl transferase, an enzyme which catalyses the prenylation of *p*-hydroxybenzoate with an all-trans polyprenyl group thus forming the polyisoprenoid side chain (Ashby et al., 1992; Forsgren et al., 2004). Relative expression and function of these genes remain to be determined in humans and specifically in the heart. Although the full molecular nature and sites responsible for de novo synthesis of CoQ_{10} are yet to be fully delineated, there is evidence that CoQ_{10} is synthesised in the endoplasmic reticulum and Golgi system (Kalen et al., 1989). Whether chronic heart disease and/or ageing impact the capacity to conduct endogenous synthesis or metabolise and distribute dietary sourced CoQ_{10} is yet to be elucidated. The capacity to augment myocardial CoQ_{10} in the disease state is crucial to successful therapeutic interventions.

8. Summary and conclusions

There is robust and increasing evidence that oxidative stress is an important contributor to the pathophysiology of cardiovascular diseases including heart failure, hypertension and ischaemic heart disease.

Despite conclusive data of the efficacy of CoQ_{10} therapy in animal models of many human diseases, the previous results of prospective randomised clinical trials while being encouraging have not been uniformly convincing. However recent meta-analyses of trials of the effect of CoQ_{10} therapy for heart failure and for hypertension are more persuasive.

Further research is indicated on the role of CoQ_{10} and other antioxidants in the treatment of the major cardiovascular diseases.

Crucial to therapeutic intervention is the further development of optimal CoQ_{10} formulations designed for maximum and rapid intracellular incorporation of CoQ_{10}. Of equal importance, but perhaps more realistic in the longer term, is the expansion of our knowledge regarding the genes and post-transcriptional mechanisms responsible for the endogenous intracellular synthesis of CoQ_{10}, particularly during the progression of disease and/or senescence. Such understanding will be crucial to the design of therapeutic agents that will intrinsically augment endogenous synthesis of CoQ_{10}.

Conclusive demonstration of the therapeutic potential of CoQ_{10} in heart disease requires the capacity to conduct well designed, multi-centre international trials. Such a task has been a major hurdle due to a relative dearth of funds from governments and industry for the support of a non-proprietary natural agent. However, despite this, in progress is a multi-national prospective randomised clinical trial of CoQ_{10} in advanced cardiac failure, the Q-symbio trial (Mortensen, 2003). This trial is an adequately powered trial to finally prove or disprove the clinical efficacy of CoQ_{10} in heart failure. The next few years should see major advances in our knowledge of the effect of CoQ_{10} in the treatment of diseases where oxidative stress is a major factor.

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Coenzyme Q$_{10}$ and statins: Biochemical and clinical implications

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Abstract

Statins are drugs of known and undisputed efficacy in the treatment of hypercholesterolemia, usually well tolerated by most patients. In some cases treatment with statins produces skeletal muscle complaints, and/or mild serum CK elevation; the incidence of rhabdomyolysis is very low. As a result of the common biosynthetic pathway Coenzyme Q (ubiquinone) and dolichol levels are also affected, to a certain degree, by the treatment with these HMG-CoA reductase inhibitors. Plasma levels of CoQ$_{10}$ are lowered in the course of statin treatment. This could be related to the fact that statins lower plasma LDL levels, and CoQ$_{10}$ is mainly transported by LDL, but a decrease is also found in platelets and in lymphocytes of statin treated patients, therefore it could truly depend on inhibition of CoQ$_{10}$ synthesis. There are also some indications that statin treatment affects muscle ubiquinone levels, although it is not yet clear to which extent this depends on some effect on mitochondrial biogenesis. Some papers indicate that CoQ$_{10}$ depletion during statin therapy might be associated with subclinical cardiomyopathy and this situation is reversed upon CoQ$_{10}$ treatment. We can reasonably hypothesize that in some conditions where other CoQ$_{10}$ depleting situations exist treatment with statins may seriously impair plasma and possible tissue levels of coenzyme Q$_{10}$. While waiting for a large scale clinical trial where patients treated with statins are also monitored for their CoQ$_{10}$ status, with a group also being given CoQ$_{10}$, physicians should be aware of this drug-nutrient interaction and be vigilant to the possibility that statin drugs may, in some cases, impair skeletal muscle and myocardial bioenergetics.
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1. Introduction

Statins have constituted an important breakthrough in the treatment of hypercholesterolemia because of their efficacy and high short-term tolerability. Long-term compliance with statin therapy is variable: whereas in the landmark secondary and primary prevention trials discontinuation rates were contained and to the same extent in the placebo and in treated groups, adherence to statin therapy found in clinical practice is lower (Jackevicius et al., 2002). These drugs reduce cardiovascular events in coronary heart disease patients with moderate and mild LDL-C elevations. Many of the beneficial effects of statins might be related also to other properties aside from the lipid-lowering effects, notwithstanding the clear relationship between reduction of mortality and LDL decrease (Baigent et al., 2005).

Even though they are usually well tolerated in the short term by most patients, statins can produce a variety of muscle-related complaints or myopathies. It is important to note that on placebo 5–10% of patients develop aches and pains and on statin therapy an extra 5% develop muscle-related symptoms. Besides skeletal muscle complaints, mild serum CK elevations, myalgia, muscle weakness, and cramps, there is a very low incidence of rhabdomyolysis, which represents a serious side effect. Expert consensus guidelines for the management of statin-related muscle complaints establish that prevention is the best approach. The lowest statin dose to achieve therapeutic goals should be used and patients should be instructed on the importance of reporting unexpected muscle pain or weakness

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or discoloration of urine to their physician. When in expert hands statins are safe drugs and the almost 100 rhabdomyolysis cases observed with cerivastatin several years ago have served to adequately instruct the medical profession on a correct and satisfactory use of these drugs.

Thompson and co-workers discussed the issue in an article (Thompson et al., 2003) where the following are indicated as the possible mechanisms of statin-induced muscle injury:

- reduction of the cholesterol content of skeletal muscle membranes,
- reduction in farnesyl pyrophosphate, an intermediary for the production of ubiquinone, which is required for the activation of small GTP-binding regulatory proteins,
- reduction of the levels of ubiquinone.

Ubiquinone (Coenzyme Q_{10} in humans) was first discovered in the mitochondrial respiratory chain, where it plays an essential role in oxidative phosphorylation. In the following years research also focused on its antioxidant properties in different cellular compartments and in plasma lipoproteins (Tomasetti et al., 1999). Newly discovered roles concern its effect on permeability transition pores and uncoupling proteins (Dallner and Stocker, 2005). The clinical aspects of coenzyme Q have recently been reviewed (Littarru and Tiano, 2005).

2. Statins and CoQ_{10} biosynthesis

The first double blind study on the effect of HMG-CoA reductase inhibitors on plasma CoQ_{10} levels was by Ghirlanda et al. (1993). The rationale of that study lay in the fact that statins also block the biosynthesis of coenzyme Q_{10} and of dolichol, besides the well-known effect on cholesterol synthesis. Even earlier Karl Folkers described a decrease of CoQ_{10} levels, following lovastatin treatment in plasma of cardiac patients (Folkers et al., 1990) as well as in plasma and in liver of rats (Willis et al., 1990). Following those initial observations, several human studies have shown that administration of different kinds of statins can lead to a parallel decrease of coenzyme Q_{10} and cholesterol in plasma (Ghirlanda et al., 1993; Folkers et al., 1990; Bargossi et al., 1994; Mortensen et al., 1997; Kaikkonen et al., 1999; De Pinieux et al., 1996; De Lorgeril et al., 1999; Human et al., 1997; Miyake et al., 1999; Watts et al., 1993; Passi et al., 2003; Laaksonen et al., 1995, 1996; Rundek et al., 2004). The initial part of the mevalonate pathway is a sequence of reactions that forms farnesyl PP (FPP) from acetyl-CoA. FPP is the last common substrate for the biosynthesis of several end products including CoQ. The branch point enzymes have different affinities for FPP and consequently, a change in the FPP pool will have different effects on the rate of biosynthesis of each lipid. There are indications that in humans (and rats) *trans*- and *cis*-prenyltransferases have higher affinity

to FPP than does squalene synthase: consequently a limited inhibition of the HMG-CoA reductase would affect squalene synthase, and therefore cholesterol biosynthesis, while the other two enzymes would still be saturated. Further inhibition of the reductase may also reduce the saturation of the other enzymes, i.e. coenzyme Q and dolichol synthesis would also be affected. Coenzyme Q amounts were decreased in rat heart, muscle and liver after treatment with the HMG-CoA-inhibitor mevinolin indicating the importance of a decreased FPP pool in CoQ biosynthesis (Low et al., 1992). Rodent studies have therefore demonstrated a partial inhibition of CoQ synthesis in several organs upon treatment with these inhibitors (Low et al., 1992).

3. Animal studies

From 1990 up to now numerous animal studies have been published involving six different animal species – seven rat studies, three hamster studies, three dog studies, one rabbit study, one guinea pig study, and one study looking at squirrel monkeys, mini pigs, and hamsters – evaluating the effect of statins on coenzyme Q blood and/or tissue levels (Willis et al., 1990; Loop et al., 1994; Satoh et al., 1995; Diebold et al., 1994; Belichard et al., 1993; Caliskan et al., 2000; Fukami et al., 1993; Ichihara et al., 1999; Marz et al., 2000; Morand et al.,1997; Nakahara et al., 1998; Pisarenko et al., 2001; Satoh and Ichihara, 2000; Sugiyama, 1998; Low et al., 1992; Schaefer et al., 2004). Ten of these 16 studies looked specifically at the adverse consequences of statin-induced CoQ depletion: decreased ATP production (Diebold et al., 1994; Caliskan et al., 2000; Pisarenko et al., 2001) increased injury after ischemia/reperfusion (Satoh et al., 1995), increased mortality in cardiomyopathy (Nakahara et al., 1998), and skeletal muscle injury and dysfunction (Fukami et al., 1993; Schaefer et al., 2004). A particularly pertinent study by Diebold et al. documented a depletion in CoQ_{10} content in heart muscle in guinea pigs when treated with lovastatin in older age animals (2 years of age), and further observed no significant depletion in CoQ_{10} content in heart muscle in the guinea pigs in the younger age group (2–4 months of age) (Diebold et al., 1994). The authors evaluated mitochondrial function as measured by the potential to phosphorylate ADP to ATP, and again documented a decrease by up to 45% in cardiac mitochondria in the 2-year-old animals treated with lovastatin, and no significant decrease in phosphorylation in the younger age group animals. This sensitivity for older animals to show clinically relevant heart muscle CoQ_{10} depletion is of concern in humans as older patients are treated with statin medications and are observed to be more fragile and more susceptible to side effects.

Animal studies to date uniformly document varying degrees of coenzyme Q depletion in blood and in tissue with statin therapy, and that the coenzyme Q deficiency is associated with adverse effects in the ischemia reperfusion injury in dog models (Satoh et al., 1995), as well as

in liver and cardiac coenzyme Q content in rabbits, causing skeletal muscle damage (Fukami et al., 1993; Nakahara et al., 1998). Significant CoQ tissue depletion was documented in the heart in hamsters upon oral administration of lovastatin (Belichard et al., 1993); in squirrel monkeys and mini pigs simvastatin administration produced a liver and heart depletion of ubiquinone (Morand et al., 1997). Moreover, it is known that in female cardiomyopathic hamsters, lovastastin treatment considerably reduces median survival time (Marz et al., 2000); the authors of that paper hypothesize that this effect may be the result of a myocardial ubiquinone depletion. It is also noteworthy that the lipid soluble statins appear to show more animal toxicity, particularly in the ischemia reperfusion dog models (Ichihara et al., 1999; Satoh and Ichihara, 2000). One can surmise from these animal studies that statins have the potential to produce clinically meaningful coenzyme Q depletion in several animal species; the inconsistent results of this depletion appear to be related to age, type of animal and dose. In all animal studies where supplemental coenzyme Q was given to the animals prior to the institution of statins, the coenzyme Q blood and tissue depletion was completely prevented.

More recently ubiquinone concentration and mitochondrial function were measured in rats treated with cerivastatin (Schaefer et al., 2004). There was a decrease of CoQ9 in muscles rich in Type II, fast twitch fibres, which was significantly different in some phases of the study.

4. Human studies

From 1990 to date many published studies in humans have evaluated the effects of statins on CoQ_{10}, mainly on its plasma levels (Ghirlanda et al., 1993; Folkers et al., 1990; Bargossi et al., 1994; Mortensen et al., 1997; De Pinieux et al., 1996; De Lorgeril et al., 1999; Human et al., 1997; Miyake et al., 1999; Watts et al., 1993; Passi et al., 2003; Laaksonen et al., 1995, 1996; Palomaki et al., 1997, 1998; Bleske et al., 2001; Jula et al., 2002; Wong et al., 2001; Rundek et al., 2004; Mabuchi et al., 2005; Paiva et al., 2005). Nine of those were controlled trials and eight of those nine studies demonstrated significant CoQ_{10} depletions secondary to statin therapy. In human trials evaluating coenzyme Q_{10} in statin therapy there appears to be frequent and significant depletion in blood CoQ_{10} levels, particularly when statins are taken at higher doses and most notably in the elderly. In one study involving patients with pre-existing CHF, the depletion in blood coenzyme Q_{10} levels was associated with a drop in ejection fraction and clinical deterioration (Folkers et al., 1990). Supplemental CoQ_{10} has been found to prevent the depletion of CoQ_{10} in blood and in one study also to prevent the depletion measured in platelet CoQ_{10} levels (Bargossi et al., 1994). The serum depletion of CoQ_{10} was associated with an elevation in lactate to pyruvate ratio, suggesting a shift toward anaerobic metabolism and possible impairment in mitochondrial

bioenergetics, secondary to statin-induced CoQ_{10} depletion (De Pinieux et al., 1996). Furthermore, two trials demonstrated enhanced oxidizability of LDL cholesterol related to the lowering of serum CoQ_{10} by statins (Palomaki et al., 1997; Palomaki et al., 1998). Supplemental CoQ_{10} has been shown to increase the CoQ_{10} content in low-density lipoproteins and to decrease significantly LDL cholesterol oxidizability (Palomaki et al., 1998). One trial found no skeletal muscle depletion of CoQ_{10} in statin treated hypercholesterolemic patients (Laaksonen et al., 1995) and one trial demonstrated no significant CoQ_{10} depletion in 12 young normolipidemic volunteers treated short term (4 weeks) with statins (Bleske et al., 2001), namely pravastatin or atorvastatin.

A study from our group monitored coenzyme Q_{10}, as well as other antioxidants in patients treated with simvastatin, atorvastatin or pravastatin. The three drugs produced a dose-dependent plasma depletion of total cholesterol and CoQ_{10} without affecting the other lipophilic or hydrophilic antioxidants. There was a depletion of CoQ_{10} also in lymphocytes (Passi et al., 2003).

Another investigation, also conducted with atorvastatin in patients at risk for cardiovascular disease and stroke, showed a decrease in CoQ_{10} plasma concentrations, which was already significant after 14 days of treatment (Rundek et al., 2004). In diabetic patients, the CoQ_{10} depletion during statin therapy appears to be associated with subclinical cardiomyopathy, with significant improvement in cardiothoracic ratios upon CoQ_{10} supplementation (Miyake et al., 1999). From these studies, one can conclude that supplemental CoQ_{10} may prevent the statin induced CoQ_{10} deficiency state without altering the cholesterol-lowering or anti-inflammatory properties of these drugs and decreasing the oxidizability of low-density lipoprotein cholesterol. Furthermore, inhibition of chemotactic migration of human monocytic THP-1 cells, a beneficial, anti-inflammatory effect showed in vitro by statins, was not inhibited by ubiquinone (Wong et al., 2001).

In a recent work by Berthold et al. (2006), subjects were treated with simvastatin and/or ezetimibe, a cholesterol absorption inhibitor which also increases endogenous cholesterol synthesis. Simvastatin and its combination with ezetimibe significantly decreased plasma CoQ_{10} levels, whereas ezetimibe monotherapy did not. There was a significant correlation between the CoQ_{10} level decrease and the decrease in total and LDL-cholesterol levels in all three treatment groups. The CoQ_{10}/total chol ratio increased significantly in all the treatment groups. The authors conclude that the CoQ_{10} decrease may simply reflect the decrease in the levels of its lipoprotein carriers. Several years ago we conducted a trial in hypercholesterolemic patients who were divided into three groups and treated with increasing dosages of atorvastatin or pravastatin or simvastatin, respectively. Whereas in the simvastatin group, both at 10 and 20 mg/day, the CoQ_{10} total cholesterol ratio remained unchanged, atorvastatin and pravastatin produced a progressive decrease of the ratio with increasing

dosages. Moreover, there was a significant decrease of CoQ_{10} content in lymphocytes: this decrease was most evident in the atorvastatin (20 mg) group, where it reached 66%, whereas it was less pronounced in the pravastatin and simvastatin groups where it was 35% and 37%, respectively.

In a recent prospective case-control study (Stocker et al., 2006) conducted with samples from the LIPID study, it was reported that neither plasma CoQ_{10} concentration, nor its decline during pravastatin therapy, was linked to recurrent cardiovascular disease events. The authors indicate that prolonged sample storage may have contributed to the found CoQ_{10} values, even though CoQ_{10} did not predict CVD events even after adjustment for storage time. Mean CoQ_{10} values were 0.60 μM for the placebo group and 0.51 μM for the pravastatin treated group. In a study already mentioned by Passi et al., the decrease in plasma CoQ_{10} after pravastatin therapy (40 mg/day, as in the lipid study) was from 1.11 to 0.6 μM.

5. Statins and muscle levels of CoQ_{10}

Even though some studies have shown a decrease of CoQ_{10} in the course of statin treatment in platelets (Bargossi et al., 1994) and in lymphocytes (Passi et al., 2003), a decrease in serum ubiquinone concentration does not always reflect a similar decrease in intracellular CoQ_{10}.

In fact an early study indicated that decreased ubiquinone concentration in patients treated with statins were accompanied by increased CoQ_{10} concentrations in skeleton muscle. (Laaksonen et al., 1995). Recent work by the same group (Paiva et al., 2005) showed that in a group of hypercholesterolemic patients treated with high doses of simvastatin (80 mg/day) muscle ubiquinone concentration underwent a significant, 30%, reduction, but no decrease was seen in the atorvastatin or placebo group. Respiratory chain enzyme activities were measured in 6 subjects taking simvastatin with markedly reduced muscle ubiquinone concentrations. Complex II, complex II + III, and complex IV were reduced in patients taking simvastatin. Interestingly 80 mg/day simvastatin increased total cholesterol concentrations in muscle tissues. The authors, while commenting that the plasma ubiquinone concentration did not correlate with the respiratory chain enzyme activities, state that this is not a useful biomarker when evaluating the effects of statins on tissue energy metabolism. The fact that citrate synthase activity was also reduced suggests that the number or volume of muscle mitochondria was diminished in these subjects.

Lamperti et al. (2005) also investigated muscle biopsy specimens from 18 patients with statin drug-related muscle symptoms or high CK levels. In two patients there was histochemical evidence of mitochondrial dysfunction. Another two patients had a few necrotic and vacuolated fibres. Although the level of muscle CoQ_{10} was not significantly different in the patients as a group, it was below 2 SDs of the normal mean in three patients and below 1 SD in seven patients. The authors conclude that statin drugs may decrease the concentration of CoQ_{10} in muscle to a modest extent in some patients. Very recently (Vercelli et al., 2006), reported that coenzyme Q_{10} levels were below 50% of control values in muscle homogenates of a patient who had been treated first with simvastatin (2 years) and then atorvastatin (2 years) and Chinese red rice upon discontinuation of the latter.

6. Ubiquinone 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 indications suggesting rapid improvement in statin induced myalgia and fatigue with supplemental CoQ_{10}. This issue is extensively discussed in a review by Koumis et al. (2004). Besides anecdotic reports, two clinical trials, conducted to evaluate the tolerability and efficacy of high-dose lovastatin in cancer patients, also tested the capacity of oral supplementation with ubiquinone (240 mg/day) to prevent or mitigate lovastatin toxicity (Thibault et al., 1996; Kim et al., 2001). In the study by Thibault, ubiquinone prophylaxis did not decrease the frequency of musculo-skeletal toxicity but significantly reduced its severity. In the study conducted by Kim et al. the authors found elevated serum CK in two patients with mild myalgia and muscle weakness, but this toxicity was almost completely reversed by ubiquinone supplementation. This study though, did not include a comparative group so it is not clear what role ubiquinone supplementation had in minimizing the frequency and severity of myopathy. Furthermore, three case reports describe the occurrence of serious muscle symptoms in the course of treatment with lovastatin or simvastatin and the resolution of symptoms upon treatment with coenzyme Q_{10}, at different dosages (Walravens et al., 1989; Lees and Lees, 1995; Chariot et al., 1993). It is difficult to assess though whether CoQ10 supplementation or discontinuation of statin treatment, or a lower dosage of statins caused the resolution of symptoms. In different kinds of muscle disease, not related to statin adverse effects, the beneficial effects of coenzyme Q_{10} supplementation have been shown to correlate with improvement in oxidative phosphorylation as monitored by NMR techniques (Nishikawa et al., 1989; Mizuno et al., 1997; Barbiroli et al., 1999).

7. Ubiquinone supplementation and mitigation of adverse statin effects in cardiac patients

Folkers et al had already described, in 1990, a few cases of cardiac patients supplemented with CoQ_{10} in whom cardiac failure worsened when lovastatin was added to their therapy. This deterioration was reversed by increasing their daily dosage of CoQ_{10} (Folkers et al., 1990).

Silver and colleagues documented systematic impairment of diastolic ventricular function in stable outpatients

being started on atorvastatin therapy for hyperlipidemia (Silver et al., 2004). 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.

In a study by Colquhoun simvastatin treatment, at 20 mg/day, was accompanied by transient decrease of left ventricular ejection fraction (LVEF) while no significant change was observed at 3 and 6 months. Patients enrolled for this study were hypercholesterolemic subjects with normal LVEF (Colquhoun et al., 2005).

Very recently a study was reported 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 effects. (Langsjoen et al., 2006). 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 though has a serious limitation, 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. We can reasonably hypothesize that in some conditions where other CoQ$_{10}$ depleting situations exist, treatment with statins may seriously impair plasma and possibly tissue levels of coenzyme Q$_{10}$. A physiological decline in tissue CoQ$_{10}$ has for instance been implicated in ageing (Kalen et al., 1989; Soderberg et al., 1990) which would make the elderly more susceptible to statin-induced CoQ$_{10}$ depletion.

8. Conclusions

To summarize, published animal and human trials indicate statin induced blood and tissue CoQ$_{10}$ depletion with adverse effects noted, particularly in settings of pre-existing CoQ$_{10}$ deficiency as seen in the elderly and in those with pre-existing heart failure. At our present state of knowledge it is first and foremost essential that prescribing physicians and patients be aware of this drug–nutrient interaction and be vigilant to the possibility that statin drugs have the potential to impair skeletal muscle and myocardial bioenergetics.

From a practical point of view, the attention could be focused not only on clinical symptoms but also on CoQ$_{10}$ plasma levels in course of statin treatment. Decrease of CoQ$_{10}$ plasma levels in patients treated with statins is often parallel to cholesterol decrease: therefore particular attention should be given to those cases where CoQ$_{10}$/chol ratio falls.

Appropriate clinical studies are needed to soundly establish whether statin treated patients or a certain category of these patients should also receive CoQ$_{10}$.

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Coenzyme Q_{10} in phenylketonuria and mevalonic aciduria

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Abstract

Mevalonic aciduria (MVA) and phenylketonuria (PKU) are inborn errors of metabolism caused by deficiencies in the enzymes mevalonate kinase and phenylalanine 4-hydroxylase, respectively. Despite numerous studies the factors responsible for the pathogenicity of these disorders remain to be fully characterised. In common with MVA, a deficit in coenzyme Q_{10} (CoQ_{10}) concentration has been implicated in the pathophysiology of PKU. In MVA the decrease in CoQ_{10} concentration may be attributed to a deficiency in mevalonate kinase, an enzyme common to both CoQ_{10} and cholesterol synthesis. However, although dietary sources of cholesterol cannot be excluded, the low/normal cholesterol levels in MVA patients suggests that some other factor may also be contributing to the decrease in CoQ_{10}. The main factor associated with the low CoQ_{10} level of PKU patients is purported to be the elevated phenylalanine level. Phenylalanine has been shown to inhibit the activities of both 3-hydroxy-3-methylglutaryl-CoA reductase and mevalonate-5-pyrophosphate decarboxylase, enzymes common to both cholesterol and CoQ_{10} biosynthesis.

Although evidence of a lowered plasma/serum CoQ_{10} level has been reported in MVA and PKU, few studies have assessed the intracellular CoQ_{10} concentration of patients. Plasma/serum CoQ_{10} is influenced by dietary intake as well as its lipoprotein content and therefore may be limited as a means of assessing intracellular CoQ_{10} concentration. Whether the pathogenesis of MVA and PKU are related to a loss of CoQ_{10} has yet to be established and further studies are required to assess the intracellular CoQ_{10} concentration of patients before this relationship can be confirmed or refuted.

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1. Introduction

Phenylketonuria (PKU; McKusick 261600) and mevalonic aciduria (MVA; McKusick 251170) are autosomal recessive disorders caused by deficiencies in the enzymes, phenylalanine 4-hydroxylase (EC 1.14.16.1) and mevalonate kinase (MK; EC 2.7.1.36), respectively. Phenylalanine 4-hydroxylase catalyses the hydroxylation of phenylalanine to tyrosine and a deficiency in this enzyme leads to an accumulation of phenylalanine in the tissues and plasma of patients (Scriver et al., 1995). MK is an enzyme in the cholesterol biosynthetic pathway (Fig. 1) which catalyses the ATP dependent conversion of mevalonic acid to 5-phosphomevalonic acid. A deficiency in MK results in markedly elevated levels of mevalonic acid in the plasma and urine of patients (Hoffmann et al., 1986). Despite numerous studies the pathogenic mechanisms involved in PKU and MVA remain to be fully elucidated (Hubner et al., 1993; Kienzle Hagen et al., 2002). Overall toxicity of accumulated phenylalanine and mevalonic acid may elicit some important pathogenic effects (Clayton, 1998). However, perturbation of other metabolites precipitated by elevated phenylalanine in PKU and a deficit in cholesterol and/or other products of the mevalonate pathway in MVA may also be contributory to the clinical progression of these disorders (Clayton, 1998; Shefer et al., 2000). In recent years a small number of studies have indicated that a deficiency in the polyisoprenoid

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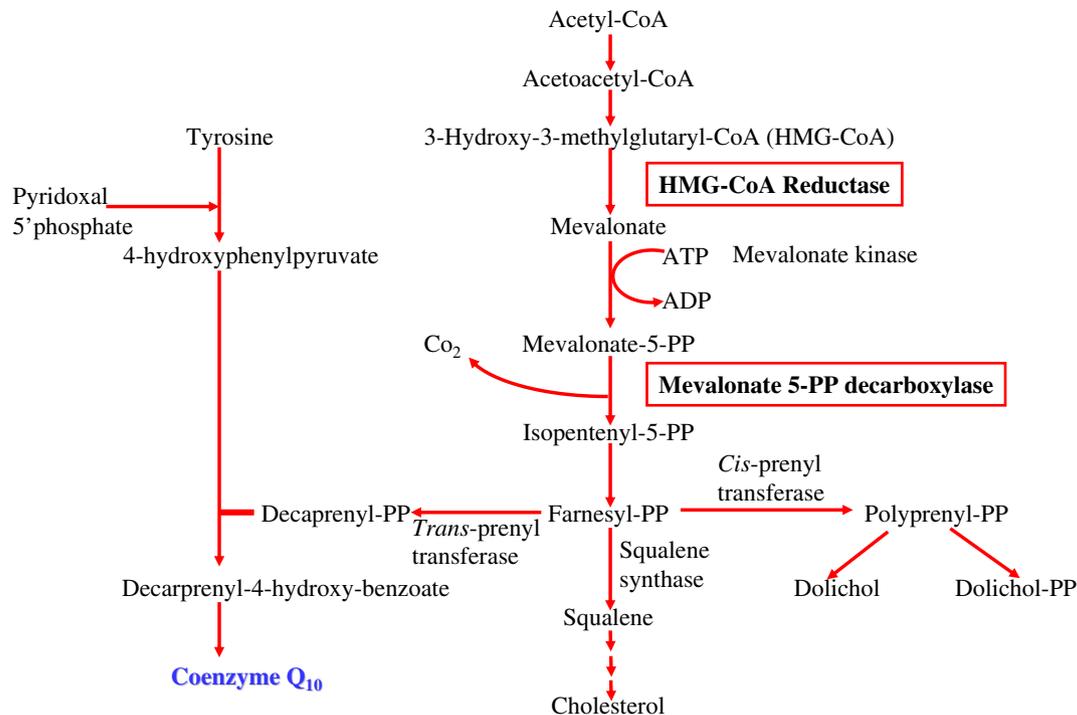
Fig. 1. The mevalonate pathway in humans illustrating the biosynthesis of CoQ10 and the enzymes sensitive to phenalylanine inhibition, HMG-CoA reductase and mevalonate 5-PP decarboxylase, □: enzyme sensitive to phenylalanine inhibition (Castillo et al., 1988).

coenzyme Q_{10} (CoQ_{10}) may also be a pathogenic factor in these disorders (Hubner et al., 1993; Artuch et al., 1999).

CoQ_{10} functions as an electron carrier in the mitochondrial electron transport chain (ETC) transporting electrons derived from complex I (NADH:ubiquinone reductase:EC 1.6.5.3) and complex II (Succinate:ubiquinone reductase:EC 1.3.5.1) to complex III (ubiquinol-cytochrome c reductase:EC 1.10.2.2) (Ernster and Dallner, 1995). It has been reported that the ETC may not be saturated with CoQ_{10}, therefore a small decrease in the level of CoQ_{10} may be sufficient to depress ATP synthesis and cause possible organ dysfunction (Estornell et al., 1992; Lenaz et al., 1997). The reduced form of CoQ_{10}, $CoQ_{10}H_2$ (ubiquinol) serves an important antioxidant role protecting cellular membranes and plasma lipoproteins against free radical induced oxidation (Takayanagi et al., 1980; Ernster and Forsmark-Andree, 1993; Alleva et al., 1997). In addition to these functions, CoQ_{10} may also play an important role in cell signalling and gene expression (Crane, 2001). Therefore in view of the electron carrier and antioxidant functions of CoQ_{10} a deficit in CoQ_{10} concentration could contribute to disease pathophysiology by causing a failure in energy metabolism as well as lowering the antioxidant capacity of the cell.

2. Mevalonic aciduria

2.1. Plasma CoQ$_{10}$ status in MVA

In humans, a MK deficiency was the first documented inborn error of CoQ_{10} biosynthesis (Hoffmann et al.,

1986; Prietsch et al., 2003). Despite residual MK activities of 0–4% of control (Hoffmann et al., 1993) cholesterol concentrations in plasma (Hoffmann et al., 1993; Hubner et al., 1993) and fibroblasts (Hoffmann et al., 1986) from patients have been found to be normal or slightly reduced and therefore a perturbation in cholesterol synthesis may not be involved in the pathophysiology of MVA. Plasma assessment has revealed evidence of a marginal CoQ_{10} deficiency (73–85% of the control mean) in MVA patients (Hoffmann et al., 1993; Hubner et al., 1993). Lowered plasma CoQ_{10} level has been attributed to liver dysfunction since hepatic synthesis is a major source of plasma CoQ_{10} (Elmberger et al., 1989), however, although hepatosplenomegaly is often associated with MVA (Hubner et al., 1993) this may not be sufficient to impair hepatic synthetic function. Evidence of decreased hepatic CoQ_{10} biosynthesis in MVA is unsubstantiated and relies upon a single report from an aborted fetus with MK deficiency who was found to have a decreased level of hepatic CoQ_{10} (69% of mean value of two aged matched controls; Hubner et al., 1993).

Radiolabelled studies have revealed low levels of CoQ_{10} biosynthetic activity (<10% of control median value) in fibroblasts from MVA patients (Hubner et al., 1993). Although in the latter experiment it was not possible to discriminate between CoQ_{10} and dolichol synthesis, the low rate of combined synthesis of these isoprenoids suggests that the failure to detect a more profound plasma CoQ_{10} deficiency in MVA patients (Hubner et al., 1993) may be the result of CoQ_{10} from dietary sources elevating the level of plasma CoQ_{10}. Dietary CoQ_{10} has a long half life (>24 h) in the circulation and can significantly

influence plasma CoQ_{10} concentrations contributing up to 25% of the total amount (Tomono et al., 1986; Weber et al., 1997).

2.2. Intracellular CoQ_{10} status in MVA

There is a paucity of information regarding the level of intracellular CoQ_{10} in MVA and these values are based only on individual assessments. Evidence of a CoQ_{10} deficiency (72% the mean value of two aged matched controls) was detected in brain tissue from the aforementioned aborted fetus with MK deficiency (Hubner et al., 1993). It was suggested that this brain CoQ_{10} deficiency may be associated with the psychomotor retardation and cerebellar ataxia observed in some MVA patients (Hubner et al., 1993). Serendipitously, one of the patient cohort examined in the study by Lamperti et al. (2003) which examined the skeletal muscle CoQ_{10} concentration of patients with cerebellar ataxia which started in childhood was found to have MVA. The skeletal muscle CoQ_{10} concentration of this patient was found to be below two standard deviations of the control mean which the authors considered indicative of a primary deficiency. Unfortunately, no further details of the patient were reported in this study. The observation of elevated plasma creatine kinase activity (a marker of muscle injury or increased sarcolemmal permeability to proteins; Smith et al., 2003) from MVA patients with decreased plasma CoQ_{10} status may add further credence to the possibility of muscle CoQ_{10} deficiency in this disorder.

2.3. Oxidative stress in MVA

Low density lipoproteins (LDL) isolated from MVA patients during episodic crisis conditions characterised by fever, nausea, diarrhea and weakness have shown increased susceptibility to oxidative modification (Hubner et al., 1993). Since ubiquinol is closely linked to vitamin E and serves to regenerative the active form of the vitamin, α-tocopherol (Constantinescu et al., 1994) the demonstration that α-tocopherol levels decreased more rapidly in LDL particles isolated from MVA patients during crisis conditions compared to controls suggested a lowered ubiquinol level in these patients (Hubner et al., 1993). Despite undergoing CoQ_{10} supplementation, a decreased ubiquinol level (6% of control median concentration) was detected in LDL isolated from an MVA patient during the crisis condition. In contrast, a normal ubiquinol status was detected in LDL from a CoQ_{10} supplemented MVA patient out of episodic crisis (Hubner et al., 1993). These results indicate that a decrease in the level of ubiquinol may be involved in the pathophysiology of the episodic crisis of MVA. No studies have yet assessed the ubiquinol level of non-supplemented MVA patients out of episodic crisis. However, the observed cataract formation in some of these patients may indicate an increased susceptibility to oxidative stress (Hubner et al., 1993; Vinson, 2006).

3. Phenylketonuria

3.1. Plasma/serum CoQ_{10} status of PKU patients

Artuch et al. (1999) were first to report low levels of CoQ_{10} in patients with PKU. In this study the serum CoQ_{10} level of dietary treated PKU patients was found to be significantly decreased compared to an age match reference population ($p < 0.01$ for patients aged 1 month < 8 years and $p < 0.00005$ for patients aged 8–33 years). The factors responsible for this deficiency were not determined however a relationship between the protein restricted diets of PKU patients and low serum CoQ_{10} level was indicated (Artuch et al., 1999). The restrictive diets of PKU patients which avoid foods such as poultry, meat, soybean products and nuts which are rich sources of CoQ_{10} (Weber et al., 1997; Kamei et al., 1986) may contribute to a lowering of serum/plasma CoQ_{10}. PKU patients on restricted diets have also been reported to have low blood levels of vitamin B6 (Schulpis et al., 2002). The active form of vitamin B6, pyridoxal 5-phosphate is required for the initial transamination of tyrosine into 4-hydroxyphenylpyruvic acid in CoQ_{10} biosynthesis (Fig. 1) and a study by Willis et al. (1999) has indicated a strong correlation between blood CoQ_{10} and vitamin B6. Therefore the possible restriction of vitamin B6 intake may also be responsible for the low serum CoQ_{10} level reported in PKU, although this factor was not investigated in the study by Artuch et al. (1999). Interestingly, the vitamin B6 intake of adult patients off dietary restriction has been reported to be below the recommended daily intake although this study did not evaluate the CoQ_{10} level of patients (Hvas et al., 2006). The availability of tyrosine is integral to the synthesis of CoQ_{10} (Fig. 1; Turunen et al., 2004) however, in PKU patients no association has been reported between the lowered plasma tyrosine concentration of patients and their serum CoQ_{10} level (Artuch et al., 2001). The membrane transport of tyrosine, phenylalanine and other large neutral amino acids (LNAA) is mediated by means of a common carrier (Smith et al., 1987). In contrast to other tissues, transport of LNAA across the blood brain barrier (BBB) is saturated at physiological plasma concentrations of LNAA (Bjerkenstedt et al., 2006). Plasma phenylalanine concentrations commonly observed in untreated PKU have been shown to impair the uptake of LNAA including tyrosine across the BBB (Oldendorf, 1973) and other neurological cell membranes (Aragon et al., 1982). Therefore the possibility arises that brain cell tyrosine levels may be limiting for CoQ_{10} biosynthesis in PKU patients, although this has yet to be investigated. Dietary intervention may be a means of correcting this putative tyrosine deficiency by lowering the blood phenylalanine concentration of PKU patients. LNAA have been used on a limited number of patients with PKU with the purpose to decrease the influx of phenylalanine into the brain. Unfortunately, due to the common carrier, LNAA treatment has the potential to create imbalances in the brain level of LNAA. However, new

amino acid formulations such as NeoPhe which contains optimum levels of all the LNAA is proving effective in the treatment of PKU (Matalon et al., 2006).

The main factor associated with the decreased serum CoQ_{10} level of PKU patients is elevated phenylalanine (Artuch et al., 2001). Experimentally induced hyperphenylalaninemia has been reported to inhibit the activities of brain and liver 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA) reductase and mevalonate-5-pyrophosphate decarboxylase by approximately 31% and 43%, respectively (Castillo et al., 1988). The concentration of phenylalanine required for this inhibition ($\geqslant 250\ \mu M$; Castillo et al., 1991) is within the physiological plasma level (108–1800 μM) of PKU patients (Artuch et al., 2001; Sirtori et al., 2005). Perturbations in cholesterol biosynthesis were demonstrated in a genetic mouse model of PKU with decreased hepatic and serum cholesterol concentrations being reported. Phenylalanine was found to noncompetitively inhibit HMG-CoA reductase, although its K_i value was not reported (Shefer et al., 2000). The phenylalanine metabolite, phenylacetate has also been shown to inhibit HMG-CoA reductase activity, however, the dose of phenylacetate required for this inhibition was vastly in excess of physiological levels (Castillo et al., 1991; Shefer et al., 2000). Therefore it was proposed that phenylacetate and other phenylalanine metabolites may not play a pathophysiological role in PKU (Shefer et al., 2000). Despite perturbations in cholesterol synthesis being demonstrated (Shefer et al., 2000), no studies have so far directly assessed the effect of hyperphenylalaninemia upon CoQ_{10} synthesis.

HMG-CoA reductase is the major regulatory enzyme in the early stages of cholesterol and CoQ_{10} biosynthesis (reviewed by Hargreaves, 2003; Fig. 1). However, control is exercised in the later stages of the mevalonate pathway (Fig. 1) by three enzymes: squalene synthase for cholesterol synthesis; *trans*-prenyl transferase for CoQ_{10} synthesis and *cis*-prenyl transferase for dolichol synthesis (Turunen et al., 2002). It is recognised that each of these enzymes has a different affinity for its substrate, farnesyl pyrophosphate. Thus an alteration in the concentration of farnesyl pyrophosphate would be expected to have a differential effect upon the rate of synthesis of the various end products: cholesterol, dolichol and CoQ_{10} (Faust et al., 1980). Squalene synthetase is reported to have the lowest affinity for farnesyl pyrophosphate, therefore a decrease in the level of this substrate would be expected to have a more profound effect upon cholesterol synthesis than the synthesis of CoQ_{10} or dolichol (Faust et al., 1980). This rationale is fundamental to the use of the HMG-CoA reductase inhibitors, 'statins' in the treatment of hypercholesterolemia (reviewed by Hargreaves et al., 2005). Therefore, although phenylalanine has been reported to inhibit cholesterol synthesis (Castillo et al., 1988; Shefer et al., 2000), this may not necessarily be accompanied by a decrease in CoQ_{10} synthesis. In an attempt to restore hepatic cholesterol levels decreased as a result of hyperphenylalaninemia (Shefer et al., 2000)

the liver may increase its uptake of LDL, the major carrier of CoQ_{10} and cholesterol in the circulation (Tomasetti et al., 1999) and this may cause a decrease in plama/serum CoQ_{10} level (Shefer et al., 2000). However, the fact that CoQ_{10} is decreased when expressed in relation to serum cholesterol levels (an indicator of total serum lipid status; Hughes et al., 2002) suggests that the lowered serum CoQ_{10} level of PKU patients may not simply reflect a decreased circulatory LDL status (Artuch et al., 1999).

3.2. Intracellular CoQ_{10} status of PKU patients

Plasma/serum CoQ_{10} has been reported to be highly dependent upon the concentration of its lipoprotein carriers and dietary intake (McDonnell and Archbold, 1996; Weber et al., 1997). Cellular CoQ_{10} concentration depends mainly upon endogenous synthesis (Ernster and Dallner, 1995) and therefore serum/plasma assessment may be of limited value as a means of assessing cellular CoQ_{10} concentration (Duncan et al., 2005). When lymphocytes were used as surrogates to assess the level of endogenous CoQ_{10} in dietary controlled PKU patients evidence of a deficit in CoQ_{10} was reported (Colome et al., 2002). In contrast, a study by Hargreaves et al. (2002), found no evidence of a CoQ_{10} deficiency in blood mononuclear cells from PKU patients on dietary restriction as well as patients off treatment. The disparity in these results was attributed to the different age group of patients studied in the two studies. In the study by Colome et al. (2002) the median age of patients was 16 years whilst the median age was 30 years in the study by Hargreaves et al. (2002). With respect to the apparently normal level of mononuclear cell CoQ_{10} biosynthesis, it was suggested that a possible metabolic adaptation had occurred in these older patients allowing normal level of CoQ_{10} synthesis to occur in the presence of hyperphenylalaninemia (Hargreaves et al., 2002). Whether this involved a decreased sensitivity of HMG-CoA reductase to hyper-phenylalaninemia inhibition (Castillo et al., 1988) remains to be established.

3.3. Oxidative stress in PKU

Oxidative stress is one of the mechanisms which has been proposed to account for pathophysiology of neurological dysfunction characteristic of PKU (Kienzle Hagen et al., 2002). Evidence of lipid peroxidation as indicated by increased plasma malondialdehyde formation has been reported in PKU patients (Sirtori et al., 2005) which is concomitant with a decreased antioxidant capacity (Sirtori et al., 2005; Van Bakel et al., 2000). Several factors have been associated with the decreased antioxidant capacity in PKU; decreased glutathione peroxidase (GSH-PX) activity (Kienzle Hagen et al., 2002); decreased catalase activity (Artuch et al., 2004); lowered CoQ_{10} status (Colome et al., 2003). Decreased GSH-PX activity was initially attributed to a selenium deficiency, (an essential cofactor of GSH-PX; Wilke et al., 1992) as a result of the restricted

diets of PKU patients (Darling et al., 1992). However, decreased GSH-PX activity in conjunction with normal plasma selenium levels has been reported (Sierra et al., 1998). Subsequent investigations in a rat model of hyperphenylalaninemia have suggested that although selenium deficiency may be contributory, inhibition of GSH-PX activity may be caused by phenylalanine directly reducing the synthesis of the enzyme and/or increasing its degradation (Kienzle Hagen et al., 2002). In this study, hyperphenylalaninemia was also found to significantly reduce catalase activity ($p < 0.01$ compared to control). In contrast, catalase activity has been shown to be only mildly decreased (7% lower than control mean; Artuch et al., 2004) or unaffected (Sirtori et al., 2005) in PKU patients. The lowered CoQ_{10} concentration of PKU patients has been associated with increased levels of both α-tocopherol consumption and malondialdehyde formation (Colome et al., 2003). In view of the suggested pro-oxidant capacity of α-tocopherol, monotherapy of this vitamin may not be appropriate to ameliorate the lipid peroxidation reported in PKU patients (Thomas et al., 1997). In contrast, joint supplementation with α-tocopherol and CoQ_{10} maybe advisable since CoQ_{10} is able to prevent the pro-oxidant activity of α-tocopherol (Thomas et al., 1996) as well as complement the antioxidant activity of the vitamin (Thomas et al., 1997; Yamamoto et al., 1990).

3.4. CoQ_{10} deficiency and tremor in PKU

It has recently been reported in an abstract by Campistol et al. (2006) that a CoQ_{10} deficiency may be involved in the pathogenesis of tremor in PKU. In a study of 41 PKU patients, 10 patients showed a high frequency (7–12 Hz) low amplitude postural and kinetic tremor. Assessment of plasma CoQ_{10} status revealed that patients with tremor had lower CoQ_{10} values than PKU patients without tremor. Neurological examination revealed no detectable tremor in two PKU patients with tremor who had under gone early stage treatment with CoQ_{10} supplementation. This clinical improvement was accompanied by an increase in plasma CoQ_{10} from a mean value of 0.21 μM (reference interval: 0.62–2.10) to 1.27 μM following supplementation (Campistol et al., 2006). In contrast, neurological examination revealed no improvement in tremor in two late treated patients.

4. Conclusion

A number of biochemical factors may contribute to the pathophysiology of MVA and PKU. In common with MVA, a decrease in CoQ_{10} concentration has been implicated as a pathogenic factor in this disease. Unfortunately, the mechanisms attributed to this deficiency have yet to be fully established. The majority of the studies that have reported a lowered CoQ_{10} level in MVA and PKU have used plasma/serum as the surrogate for this investigation. Since plasma/serum CoQ_{10} is influenced by diet as well as its lipoprotein content it may not truly reflect intracellular CoQ_{10} concentration which depends mainly upon *de novo* synthesis (reviewed by Hargreaves, 2003). Therefore, before it can be accurately established whether a deficit in CoQ_{10} concentration is involved in the pathogenesis of these diseases further studies should be undertaken to assess the intracellular CoQ_{10} level of patients.

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Contact Daniel Brethes, President of GFB
brethes@ibgc.u-bordeaux2.fr

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Peter Csermely, Chairman of Organizing Committee
www.stress07.com
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