A BIOENERGETIC RATIONALE FOR COENZYME Q_{10} SUPPLEMENTATION TO RELEAVE DEFECTIVE MITOCHONDRIAL RESPIRATION

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Highlights: *There is accumulated evidence that efficient electron transfer in the mitochondrial respiratory chain is largely dependent on CoQ_{10} associated in stoichiometric ratios within functional supercomplexes; this raises doubts on the efficacy of exogenous CoQ_{10} supplementation in raising mitochondrial respiration and prompts the idea that the beneficial effects of CoQ_{10} be due to other roles of the quinone. Here we discuss a possible reason why exogenous CoQ_{10} may enhance defective respiration and we therefore provide support for the bioenergetic function of CoQ_{10} supplementation besides its other roles.*

Contrary to the random organization model of the respiratory chain, which was the prevailing view in the last decades of the past century (Hackenbrock et al 1986), evidence has now accumulated that a large proportion of the mitochondrial respiratory complexes in a variety of organisms is arranged in supramolecular assemblies called supercomplexes or respirasomes (Schagger and Pfeiffer, 2000; Acín-Pérez et al., 2008; Lenaz and Genova, 2010) (Fig. 1).

The natural assembly of the respiratory complexes I, III, and IV into supramolecular stoichiometric entities, such as $I_1III_2IV_{0-4}$, is not just a mere structural feature but has deep functional implications on the properties of the respiratory chain (reviewed in Lenaz and Genova, 2010).

Within the mammalian supercomplexes, Complex I is almost totally associated with Complex III, and molecules of Coenzyme Q (CoQ) are present in the lipid boundary between the two complexes. This condition strongly supports the hypothesis that CoQ bound to supercomplexes participates in electron transfer by direct substrate channeling from Complex I to Complex III, providing kinetic advantage for electron transport, whereas it seems to exclude a role for the CoQ pool, present in large excess in the lipid membrane, in physiological electron transfer between the same two enzyme complexes (see Lenaz et al., 2016 for a recent review on evidence of CoQ compartmentation and respiratory rate advantage by CoQ channeling). Surprisingly, strong evidence exists that NADH-cytochrome c oxidoreductase activity still follows Michaelis-Menten (hyperbolic) kinetics with respect to CoQ in the presence of the supercomplex I_1III_2, suggesting that an intricate relation exists between electron transfer rate and CoQ concentration in the lipid membrane. Direct titration of CoQ-depleted mitochondria reconstituted with different CoQ_{10} supplements yielded a K_m (CoQ concentration yielding half-maximal velocity) of NADH oxidation for Q_t (total CoQ_{10}, reduced plus oxidized form) in the range of 2-5 nmol/mg mitochondrial protein (Estornell et al., 1992), corresponding to 4-10 mM in the lipid bilayer. The K_m for CoQ_{10} of NADH-cytochrome c oxidoreductase was found to be much higher than that of succinate-cytochrome c oxidoreductase. A similar study on proteoliposomes containing mitochondrial Complex I and Complex III showed that the experimental rate of NADH-cytochrome c oxidoreductase was hyperbolically related to the content of CoQ_{10}, with an apparent K_m in the same range as in mitochondria (Lenaz et al., 1999).

It is worth noting that if electron transfer in the supercomplex is operated through CoQ channeling, we expect a stoichiometric behaviour of rate vs. CoQ concentration, within the concentration limits imposed by the amounts of the two complexes, whereas the experimentally observed saturation kinetics (i.e. hyperbolic behaviour) seem to contradict this requirement (Fig. 2). Nonetheless, a careful reasoning allows us to conclude that free CoQ in the pool is still necessary for proper substrate channeling within the supercomplex I1III2. In fact, as a unifying model, we can assume that the CoQ molecules bound in the respiratory supercomplex must be in a dissociation equilibrium with the CoQ pool, so

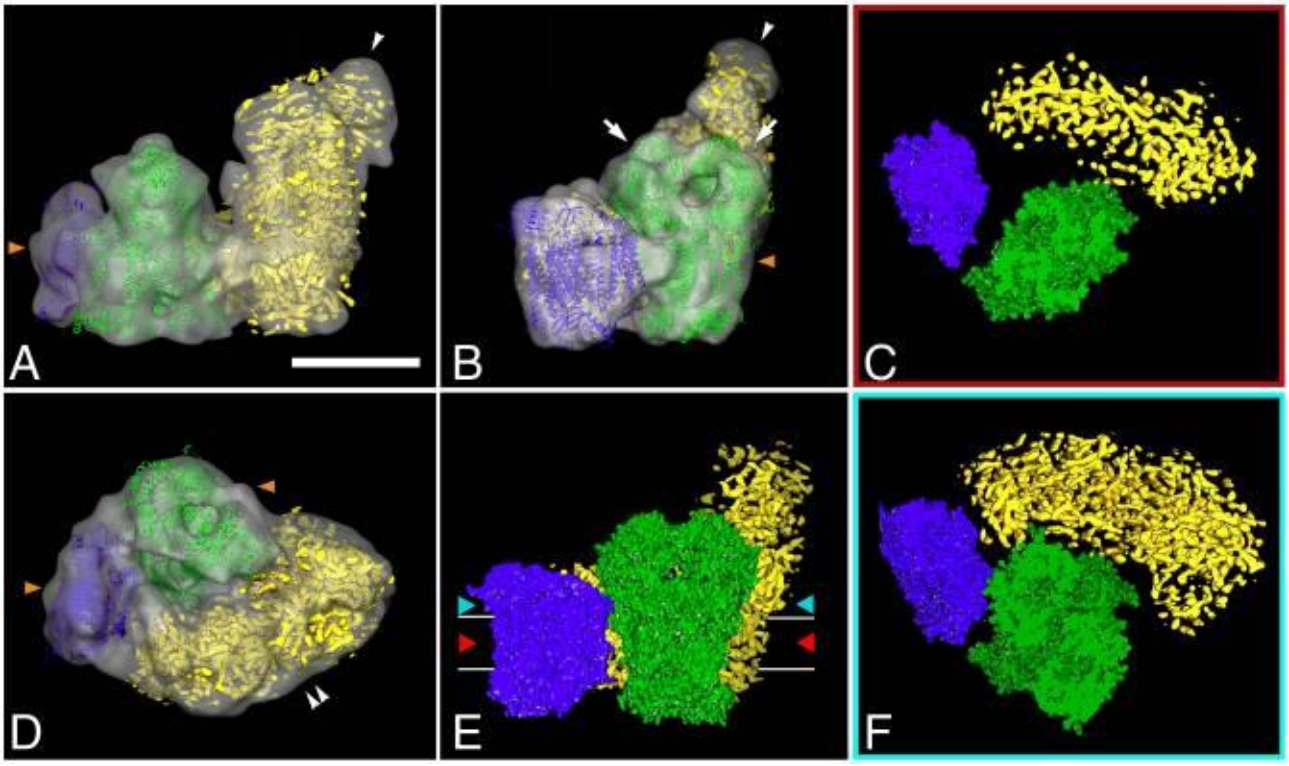

Figure 1 – Fitting of the high- and medium-resolution structures of complexes I, III_2, and IV to the 3D cryo-EM map of $I+III_2+IV$ supercomplex. (A) side view, arrowhead points to flavoproteins; (B) side view from the membrane, arrows point to core I and II subunits of complex III_2, arrowhead to flavoproteins; (C) section through the space-filling model of respirasome on the level of membrane, demonstrating gaps between complexes within the supercomplex; (D) top view from the intermembrane space, double arrowhead points to the bend of complex I in membrane; (E) space-filling model of respirasome seen from the membrane, red and light-blue arrowheads show the level of sections in C and F; (F) section through the space-filling model of respirasome on the level of matrix. In green, X-ray structure of the bovine dimeric complex III; in purple, X-ray structure of bovine monomeric complex IV; in yellow, the density map of complex I from Yarrowia lipolytica. Horizontal lines on E indicate the position of the membrane. Orange arrowheads on A, B, and D point to the position of detergent micelles. Scale bar: 10 nm. Image taken from Dudkina et al., 2011.

that their amount, at steady state, would be dictated by the size of the pool. This equilibrium may explain the saturation kinetics exhibited by the NADH-cytochrome c oxidoreductase activity in the above mentioned studies.

Our proposition also requires that the dissociation rate constants (k_{off}) of bound CoQ be considerably slower than the rates of inter-complex electron transfer via the same bound quinone molecules, in order to guarantee their compartmentation within the supercomplex and the efficient channeling of electrons (Lenaz and Genova, 2010; Genova and Lenaz, 2011) (Fig. 3).

The observation by Schneider et al. (1982) that dilution of the inner membrane proteins with phospholipids lowers electron transfer and that the effect is reversed by CoQ addition is in line with our unifying model. Earlier studies by Heron et al. (1978) also reported that endogenous CoQ_{10} leaks out of the supercomplex I_1III_2 when extra phospholipid is present, causing a decrease in redox activity that could be alleviated by adding more ubiquinone. It is likely that the function of the large amount of ubiquinone in the natural membrane may be, therefore, to maintain the proper CoQ_{10} content in the supercomplex unit when it is formed.

Analysis of the literature shows that the physiological CoQ content of several types of mitochondria is in the range of 2-4 nmol/mg protein and, therefore, only half-saturating for NADH oxidation activity (Battino et al., 1990). This implies that we would enhance the respiration rate if we further increase the content of CoQ in the inner mitochondrial membrane.

It is clear nowadays that CoQ_{10} supplementation is able to restore mitochondrial respiration in primary CoQ_{10} deficiencies and is

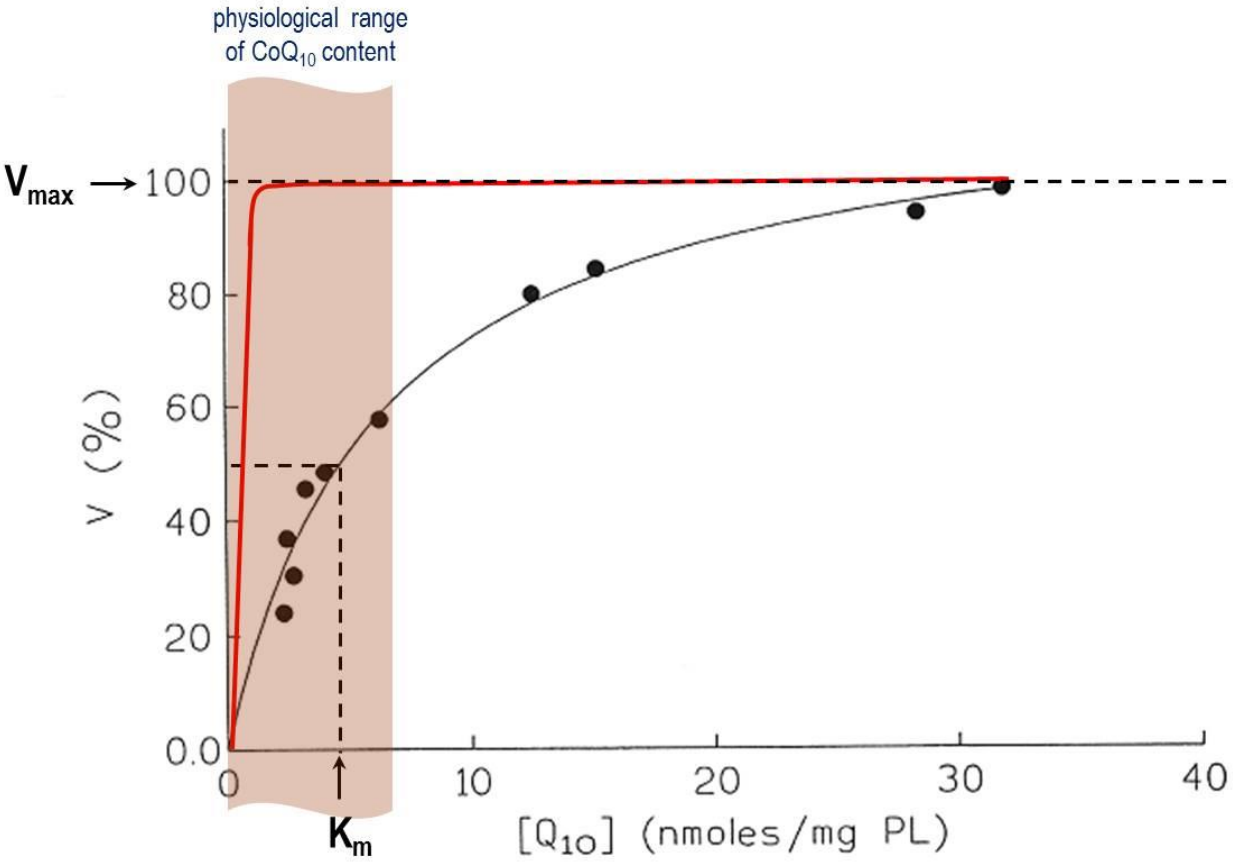

Figure 2 – Stoichiometric behaviour vs. pool behaviour. In a fixed assembly composed by Complex I and Complex III (i.e. supercomplex I_1III_2) containing bound CoQ_{10} in dissociation equilibrium with the CoQ_{10} pool of the lipid bilayer, the amount of bound CoQ_{10} is dictated by the size of the pool and the rate of NADH-cytochrome c oxidoreductase follows a hyperbolic curve (*black curve*) that increases monotonically up to the saturation plateau (Michaelis-Menten kinetics). This resembles the curve of a two-enzyme model where Complex I and Complex III are randomly distributed in the membrane, and the rate of NADH-cytochrome c oxidoreductase depends on the number of useful collisions of free CoQ_{10} molecules with their partner enzymes (pool behaviour). On the contrary, in a theoretical model (*red curve*) of a supercomplex I_1III_2 with extremely high affinity for CoQ_{10}, the integrated activity of NADH-cytochrome c oxidoreductase is quasi-proportional (linear kinetics) to the content of bound CoQ_{10} up to the saturation plateau, which would be obtained with a concentration of CoQ_{10} stoichiometric to the content of the redox components in the supercomplex and therefore very much lower than the physiological CoQ_{10} concentration. In this theoretical case, the excess of CoQ_{10} pool would exert no function (cf. text for details).

beneficial in several diseases in which a secondary CoQ_{10} deficiency is usually postulated. In this latter case, a bioenergetic deficit is not always apparent, and the health improvements are often ascribed to other properties of the quinone, such as its antioxidant activity.

Is the existence and role of supercomplexes compatible with the interpretation on bioenergetic grounds of the beneficial effects of orally administered exogenous CoQ_{10}? A major problem of CoQ_{10} administration is its low bioavailability due to its extreme hydrophobicity (Beg et al., 2010). A water-soluble formulation has recently been shown to be easily incorporated into cultured cells and their mitochondria, enhancing respiration and antioxidant properties (Bergamini et al., 2012). The same formulation was found to improve grip strength and to inhibit apoptosis in aged rats (Xu et al., 2010).

Ignoring the above mentioned notion that bound CoQ is in a chemical equilibrium with CoQ in the membrane pool, would make the existence of respiratory supercomplexes, where only bound CoQ is active, apparently incompatible with a dose-dependent effect of added CoQ_{10}. On the contrary, our interpretation supports the idea that even a slight decrease of endogenous CoQ_{10} content in the membrane is sufficient to dissociate

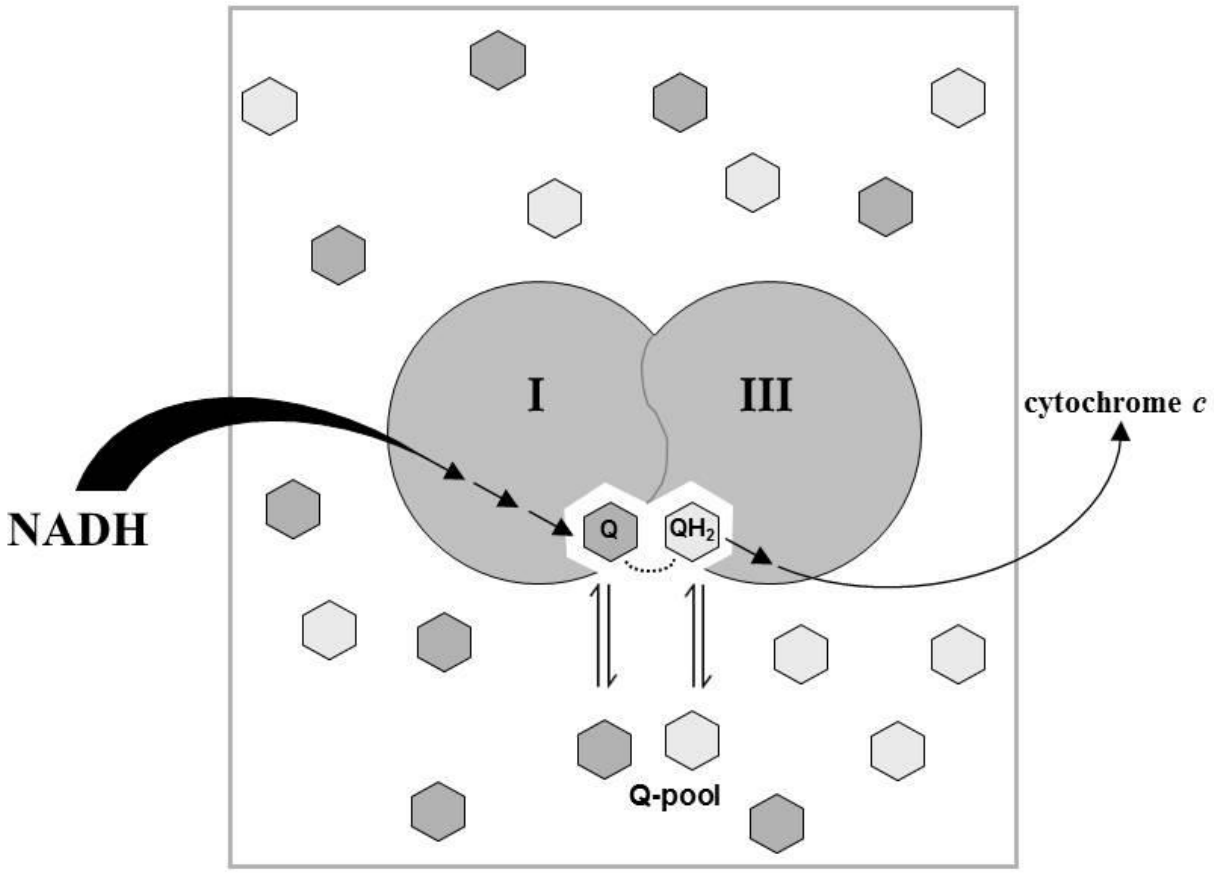

Figure 3 – Possible mechanism for CoQ_{10} molecules bound to the supercomplex I_1III_2 in dissociation equilibrium with the CoQ_{10} pool. Kinetic evidence for substrate channeling within the supercomplex I_1III_2 requires the dissociation rate constants of ubiquinone (Q) and ubiquinol (QH_2) to be considerably slower than the rates of electron transfer via the same quinone molecules bound to the supercomplex.

part of the quinone molecules from the supercomplex, with the obvious consequence of decreasing the rate of electron channeling.

In such a situation, increasing CoQ concentration in the membrane is bound to enhance the respiratory activity. This is indeed what was found in the study with the water-soluble formulation quoted above (Bergamini et al., 2012).

The fact of endogenous CoQ_{10} not being saturating in NADH oxidation should also drive a further consequence of improving respiration even when the bioenergetic defect is due to reasons different from CoQ deficiency, e.g. a defect of Complex I. This is schematically shown in Fig 4.

Indeed, a direct study by Estornell et al. (1997) demonstrated that a Complex I failure in vitamin A-deficient rats could be counteracted by an increase of CoQ_{10} after exogenous supplementation. We think that the unifying model of bound/pool CoQ described in this paper is the most likely explanation why CoQ_{10} supplementation was found to ameliorate several disease states even if a CoQ deficiency was not demonstrated.

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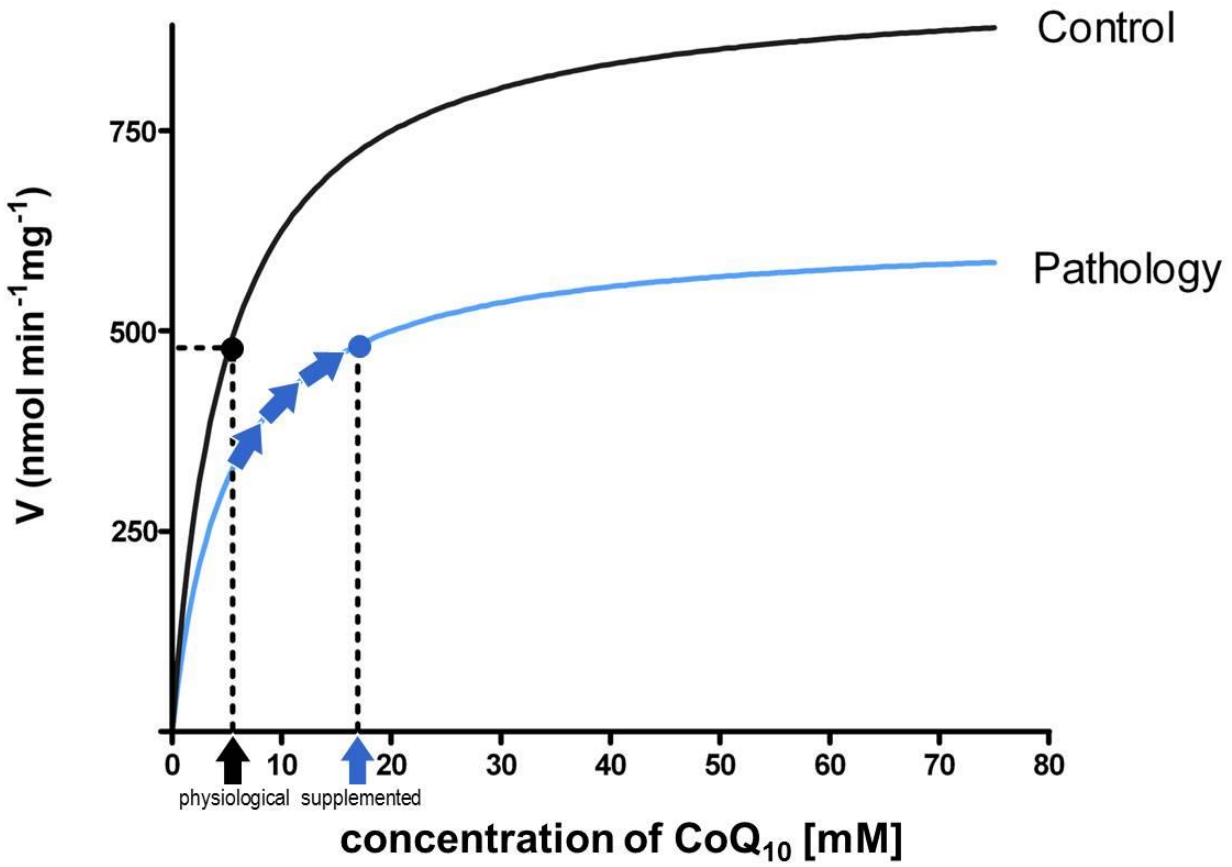

Figure 4 – Coenzyme Q_{10} supplementation restores impaired NADH oxidation. Being the physiological content of CoQ10 in the first order arm of the curve, an increase of CoQ_{10} concentration is bound to enhance NADH oxidation both in normal mitochondria (*black*) and in mitochondria with defective respiration impaired by causes different from CoQ deficiency (*blue*).

References

Acín-Peréz, R., Fernández-Silva, P., Peleato, M.L., Pérez-Martos, A., and Enriquez, J.A. (2008). Respiratory active mitochondrial supercomplexes. Mol. Cell *32*, 529–539.

Battino, M., Ferri, E., Gorini, A., Villa, R.F., Rodriguez Huertas, J.F., Fiorella, P., Genova, M.L., Lenaz, G., and Marchetti, M. (1990). Natural distribution and occurrence of coenzyme Q homologues. Membr Biochem *9*, 179–190.

Beg, S., Javed, S., and Kohli, K. (2010). Bioavailability enhancement of coenzyme Q10: an extensive review of patents. Recent Pat Drug Deliv Formul *4*, 245–255.

Bergamini, C., Moruzzi, N., Sblendido, A., Lenaz, G., and Fato, R. (2012). A water soluble CoQ10 formulation improves intracellular distribution and promotes mitochondrial respiration in cultured cells. PLoS ONE *7*, e33712.

Dudkina, N.V., Kudryashev, M., Stahlberg, H., and Boekema, E.J. (2011). Interaction of complexes I, III, and IV within the bovine respirasome by single particle cryoelectron tomography. PNAS *108*,15196–15200.

Estornell, E., Fato, R., Castelluccio, C., Cavazzoni, M., Parenti Castelli, G., and Lenaz, G. (1992). Saturation kinetics of coenzyme Q in NADH and succinate oxidation in beef heart mitochondria. FEBS Lett. *311*, 107–109.

Estornell, E., Tormo, J.R., and Barber, T. (1997). A deficiency in respiratory complex I in heart mitochondria from vitamin A-deficient rats is counteracted by an increase in coenzyme Q. Biochem Biophys Res Commun *233*, 451–454.

Genova, M.L., and Lenaz, G. (2011). New developments on the functions of coenzyme Q in mitochondria. Biofactors *37*, 330–354.

Hackenbrock, C.R., Chazotte, B., and Gupte, S.S. (1986). The random collision model and a critical assessment of diffusion and collision in mitochondrial electron transport. J Bioenerg Biomembr *18*, 331–368.

Heron, C., Ragan, C.I., and Trumpower, B.L. (1978). The interaction between mitochondrial NADH-ubiquinone oxidoreductase and ubiquinol-cytochrome c oxidoreductase. Restoration of ubiquinone-pool behaviour. Biochem J *174*, 791–800.

Lenaz, G., and Genova, M.L. (2010). Structure and organization of mitochondrial respiratory complexes: a new understanding of an old subject. Antioxid Redox Signal *12*, 961–1008.

Lenaz, G., Fato, R., Di Bernardo, S., Jarreta, D., Costa, A., Genova, M.L., and Parenti Castelli, G. (1999). Localization and mobility of coenzyme Q in lipid bilayers and membranes. Biofactors *9*, 87–93.

Lenaz, G., Tioli, G., Falasca, A.I., and Genova, M.L. (2016). Complex I function in mitochondrial supercomplexes. Biochim Biophys Acta *1857*, 991-1000.

Schägger, H., and Pfeiffer, K. (2000). Supercomplexes in the respiratory chains of yeast and mammalian mitochondria. Embo J *19*, 1777–1783.

Schneider, H., Lemasters, J.J., and Hackenbrock, C.R. (1982). Lateral diffusion of ubiquinone during electron transfer in phospholipid- and ubiquinone-enriched mitochondrial membranes. J Biol Chem *257*, 10789–10793.

Xu, J., Seo, A.Y., Vorobyeva, D.A., Carter, C.S., Anton, S.D., Lezza, A.M.S., and Leeuwenburgh, C. (2010). Beneficial effects of a Q-ter based nutritional mixture on functional performance, mitochondrial function, and oxidative stress in rats. PLoS ONE *5*, e10572.

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