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An Energetic View: Mitochondrial Nutrition for Fatigue, the Brain, and Healthy Ageing

Dr. Iain Hargreaves

Mitochondrial and Metabolic Dysfunction in Ageing and Age-Related Diseases

11:15-12:00pm

An event by: Nutritional Medicine Institute
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Mitochondrial and metabolic dysfunction in aging and age related diseases

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Declarations:

- No relevant declaration of interest to declare.

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Aims

- Mitochondrial respiratory chain (MRC)- function and source of cellular Reactive oxygen species (ROS)
- Assessment of oxidative stress (OS)
- Coenzyme Q10 (CoQ10) – cellular function and its involvement in disease
- Cellular model of Parkinson`s disease (PD) to assess evidence of 2ndary CoQ10 deficiency and OS in disease pathophysiology
- CoQ10 supplementation and its transport across the blood brain barrier (BBB) and the effect of a CoQ10 deficiency on this transport

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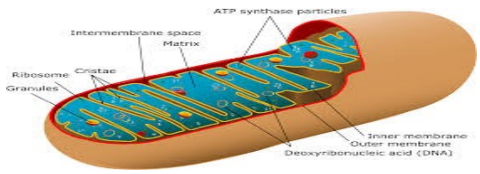
Aging and mitochondrial function

- Ageing is accompanied by a progressive loss of cellular function and systemic deterioration of multiple tissues, leading to impaired function and increased rates of morbidity
- Although the underlying mechanisms of aging have yet to be fully elucidated mitochondrial dysfunction and increased reactive oxygen species (ROS) production are thought to be important contributory factors
- The ageing process is associated with an increased risk of development and progression of the neurodegenerative disorders, Alzheimer's disease and Parkinson's disease (PD) which have also been associated with mitochondrial dysfunction and OS.



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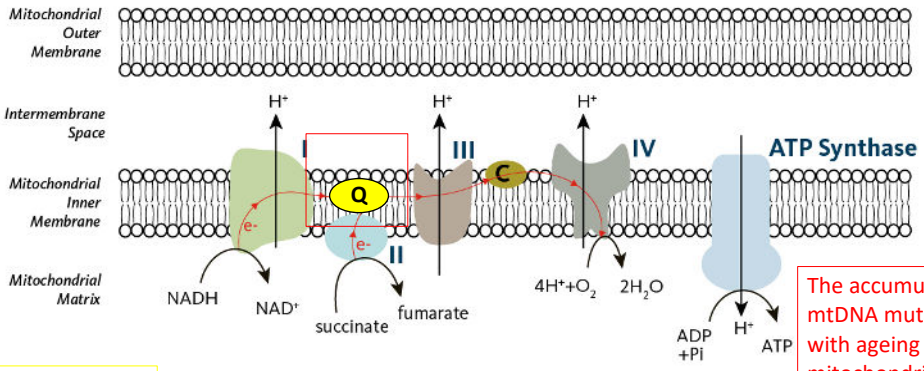
Mitochondria

- Intracellular powerhouse in which perform important cellular reactions, including the production of energy through the mitochondrial respiratory chain (MRC)

CoQ_{10} transfers electrons to complex III in MRC, essential for ATP production



Q: CoQ10

Coenzyme Q_{10} is a lipid-soluble component of the mitochondrial inner membrane that is critical to electron transport (in red)

The accumulation of somatic mtDNA mutations that occur with ageing leads to a loss of mitochondrial function.

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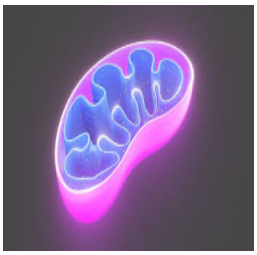
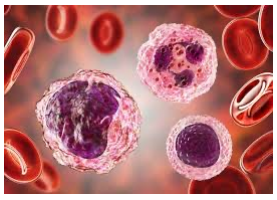
Assessing evidence of 2ndary mitochondrial dysfunction

- Secondary MRC dysfunction - consequence of aging, disease pathophysiology as well as from drug toxicity



- The gold standard for the assessment of MRC enzyme activities is a skeletal muscle biopsy but the use of low invasive surrogates may be more appropriate:
- Blood mononuclear cells or platelets which have been used to assess systemic MRC dysfunction in multiple sclerosis + PD, respectively.
- MRC dysfunction may in some cases lead to an increase expression of the hormone-like cytokine, FGF-21 which can be determined in plasma



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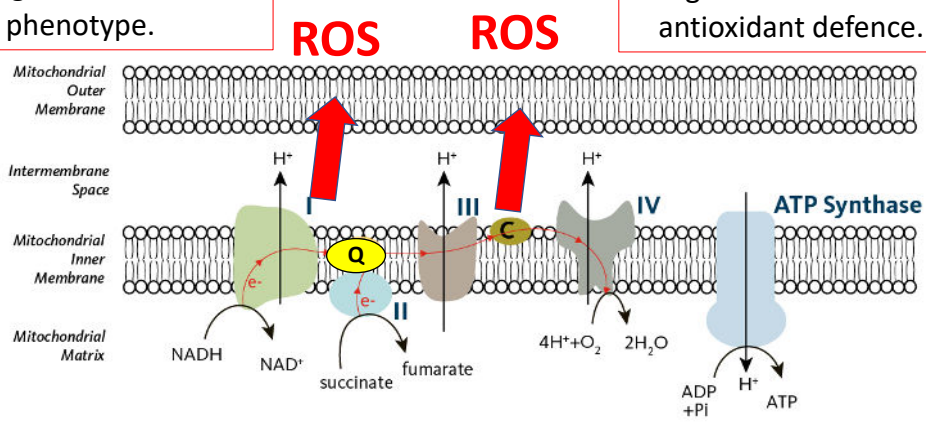
Mitochondria

- May cause oxidative damage to organelle membranes, DNA, lipids, and proteins. This damage contributes to the aging phenotype.

- During the aging process, there is a decline in ATP production and elevated ROS production together with a decline in the antioxidant defence.



ROS: Reactive oxygen species

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Assessing evidence of oxidative stress

- OS is an imbalance of free radicals and antioxidants in your body that leads to cell damage
- No single parameter has yet been recommended as a gold standard for determining evidence of OS
- 2 major methods recommended for clinical assessment using either blood or urine samples (low invasive surrogates):
- Detection of the resulting oxidative damage to biomolecules (DNA, lipids, and proteins; 8-hydroxydeoxyguanasine, isopropanes/malondialdehyde + protein carbonyls, respectively)
- Determination of antioxidant status (enzymatic antioxidant activities, non-enzymatic antioxidant levels, or total antioxidant capacity).

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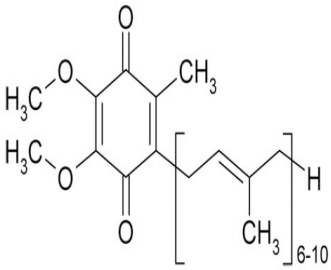
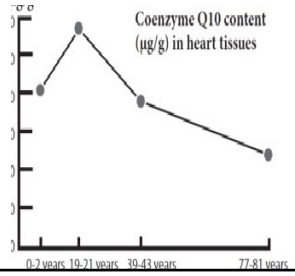
Coenzyme Q10

- CoQ10 is a member of a group of molecules known as ubiquinones which are found in animals, plants and microorganisms –ubiquitous
- 1St isolated in Liverpool in 1950`s
- Predominant ubiquinone in man is CoQ10
- Synthesized in all cells of the body apart from red blood cells- Not a VITAMIN!!!
- The endogenous production of CoQ10 decreases after the age of 20, and the myocardial concentration of CoQ10 is reduced to about half at the age of 80
- At present, there is no comparable data for changes in CoQ10 levels in the human brain as a function of normal ageing





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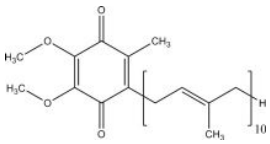
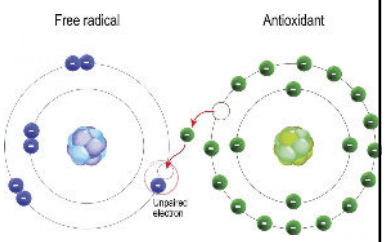
Coenzyme Q10

A decrease in synthesis of CoQ10 with aging may contribute to a loss of MRC function + OS in view of its antioxidant function



CoQ10

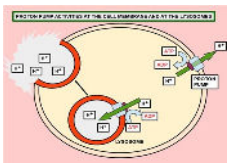


Participate in ATP production

Maintain Lysosomal pH

Acts as a powerful antioxidant in its fully reduced ubiquinol form

- Lysosome requires acidic environment for intracellular digestion .
- ATP is required for the functioning of proton pumps to maintain this acidity
- Reduction in CoQ10 -> dysfunction of the lysosome



- Scavenges free radicals that can damage cellular components such as proteins, lipids, and DNA.

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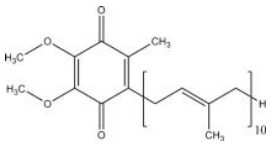
Coenzyme Q10



- In view of its roles as an antioxidant and electron carrier in the MRC a deficiency in CoQ10 status can compromise cellular antioxidant capacity and energy generation.
- As well as aging, a deficiency in CoQ10 status can result from either:
- *A genetic defect in the CoQ10 biosynthetic pathway, known as a* ***primary deficiency***
- *Result of diseases that do not result from a genetic defect in the CoQ10 biosynthetic pathway. The latter causes of a CoQ10 deficiency are known as* ***secondary deficiencies***
- Secondary CoQ10 deficiencies have been reported a range of diseases including the neurodegenerative diseases, PD and multiple system atrophy contributing to the mitochondrial dysfunction and oxidative stress documented in these disorders.

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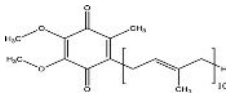
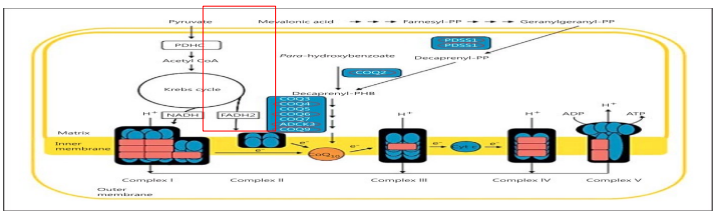
Coenzyme Q10



- In PD evidence of a CoQ10 deficiency have been reported in, plasma, platelets, plasma and cerebral cortex (post mortem study) of patients.
- CoQ10 can be determined in CSF (cerebrospinal fluid) using LC-MS analysis, although no studies have yet been undertaken in PD patients possibly due to the invasive nature of this investigation
- The cause of the CoQ10 deficiency in PD is uncertain but may result from OS induced inhibition of the biosynthesis pathway enzymes or increased degradation of CoQ10.



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Parkinson`s disease

- PD is the second most prevalent neurodegenerative disorder and the most common movement disorder affecting the current ageing population
- Characterised by a triad of motor symptoms (bradykinesia, rigidity, and tremor) and in the later stages, cognitive dysfunction, depression, and behavioural and learning disorders
- PD is characterised by a loss of dopaminergic neurons within the substantia nigra pars compacta.
- Although the aetiology of PD has not yet been fully elucidated it can result from both genetic and environmental risk factors. However, the biggest risk factor for PD is increasing age, with the median age of onset being 60 years of age

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Parkinson`s disease

- Mitochondrial dysfunction and OS are thought to be important pathophysiological factors in the disease progression.
- Mitochondrial dysfunction centred at complex I of MRC has been well documented in PD patients + animal models of the disease:

- Point mutations and deletions in the mitochondrial DNA of PD
- Impairment in iron metabolism affecting the assembly of the iron–sulphur prosthetic groups of the MRC
- The ability of α-synuclein to interact with the inner mitochondrial membrane and potentially inhibit the activity of the MRC enzymes
- Exposure to the common agricultural pesticide, rotenone

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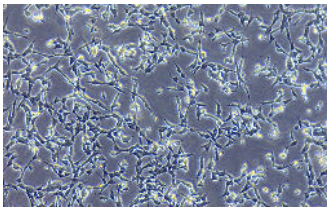
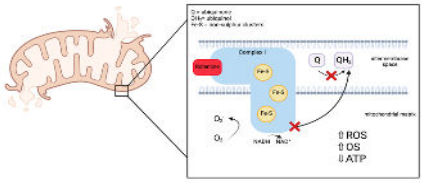
Cellular model of Parkinson`s disease

Aims: Given the association between a MRC complex I deficiency and PD does a complex I deficiency contribute to 2ndary CoQ10 deficiency and OS in disease pathophysiology

Objectives:

- Using rotenone treated SH-SY5Y human neuroblastoma cells to create a neuronal cell model of PD.
- Investigate the effect of a complex I deficiency on MRC function, cellular antioxidant status (CoQ10 + reduced glutathione (GSH) + cellular OS)





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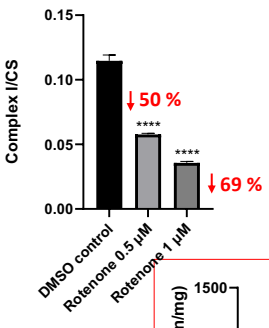
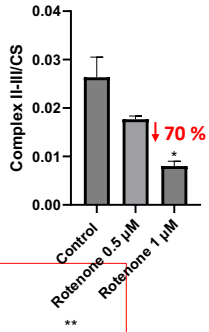
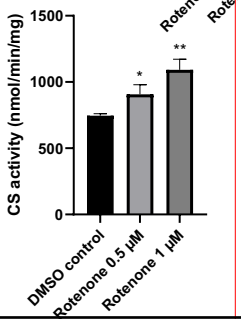
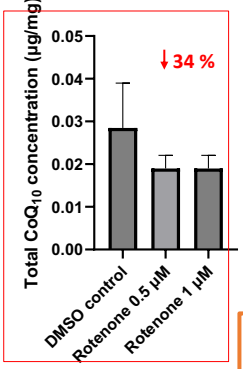
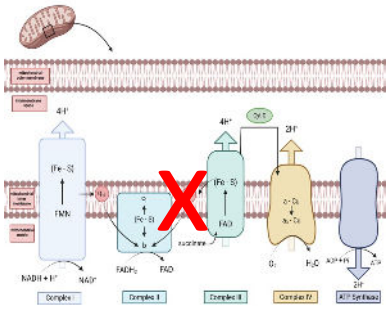
MRC enzyme activities and neuronal CoQ10 status











Cellular CoQ10 content and complex II-III activity was decreased in association with a Complex I deficiency.

Complex I deficiency resulted in significant decrease in the Complex II-III activity

Citrate synthase (CS) activity, a mitochondrial marker enzyme, was increased as a result of a complex I deficiency

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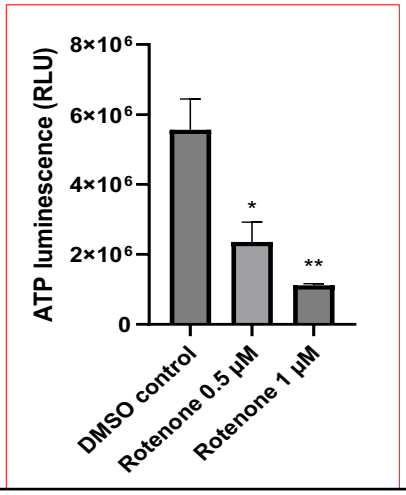
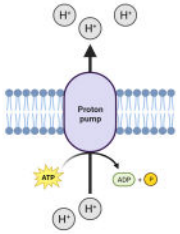
Neuronal ATP status





A significant decrease in ATP luminescence was detected MRC complex deficiency

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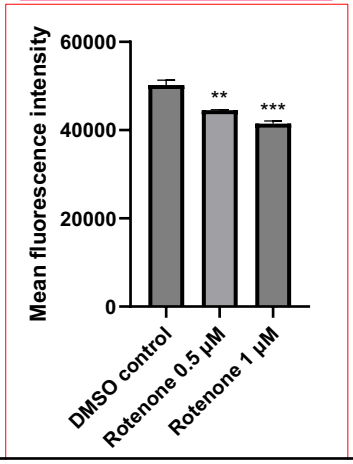
Cellular reduced glutathione status

A reduction in fluorescence intensity is directly proportional to a decrease in intracellular GSH status.



- GSH is a major intracellular thiol-dependent antioxidant
- GSH depletion thought to be an early common event in PD
- GSH deficiency may be consequence of decreased ATP availability (required for GSH biosynthesis) or is due to increased ROS levels.

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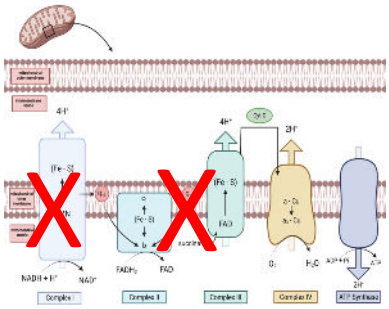
Mitochondrial dysfunction in Parkinson`s disease

? What is the effect of a deficiency in the activities of MRC complex I + II-III and a diminution in GSH + CoQ10 status on cellular ROS levels + OS.



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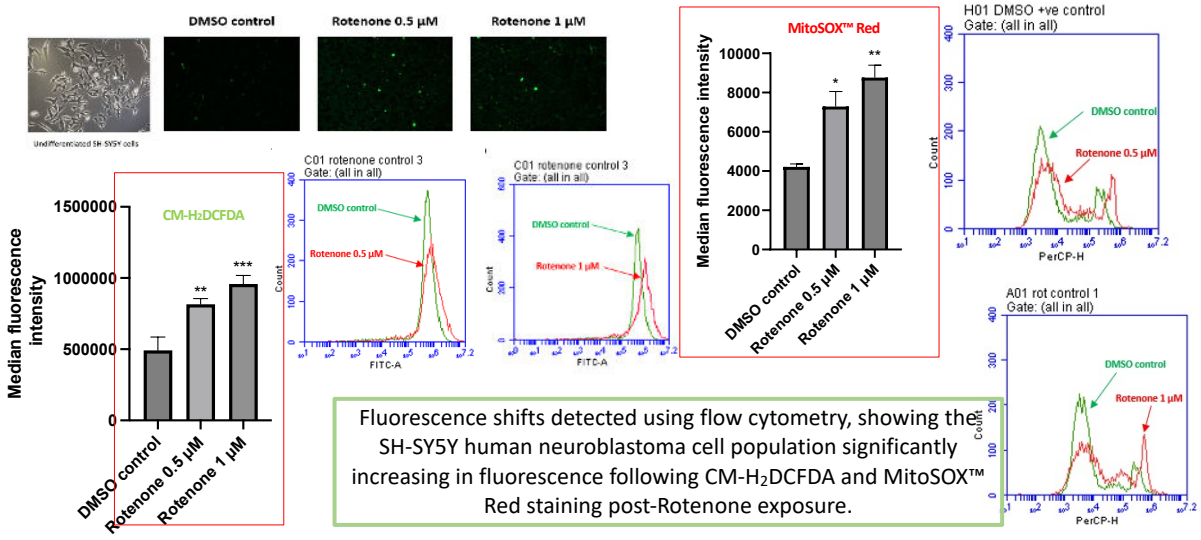
Cellular Reactive Oxygen Species Levels



Fluorescence shifts detected using flow cytometry, showing the SH-SY5Y human neuroblastoma cell population significantly increasing in fluorescence following CM-H_2DCFDA and MitoSOX™ Red staining post-Rotenone exposure.

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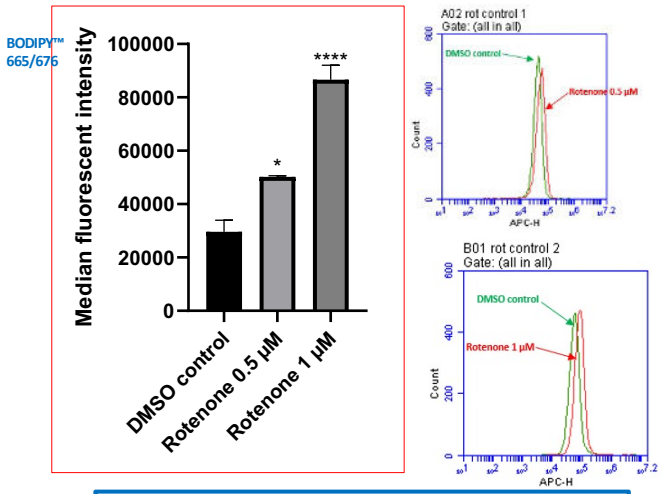
Cellular lipid peroxidation levels



Fluorescence shifts detected using flow cytometry, showing the SH-SY5Y human neuroblastoma cell population significantly increasing in fluorescence following BODIPY™ 665/676 staining post-Rotenone exposure.

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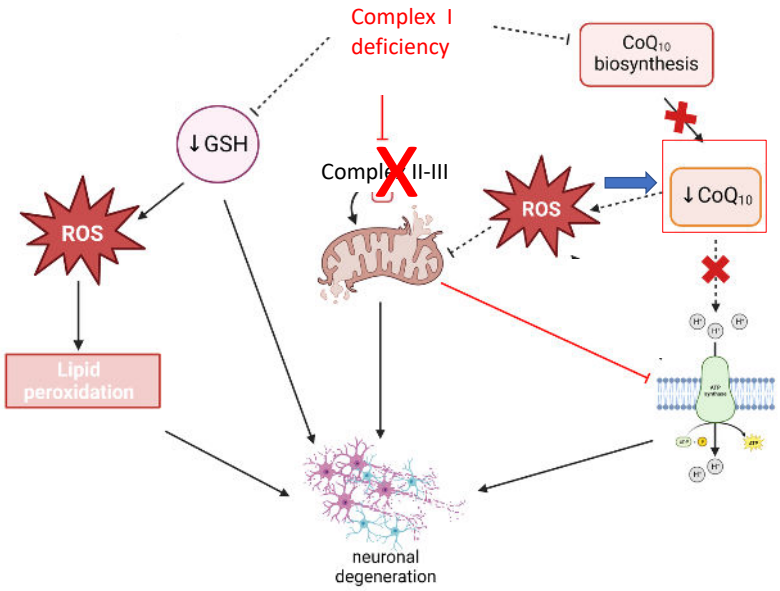
Putative Pathophysiology of Parkinson's Disease



- Reported elevated ROS may occur from MRC complex I deficiency
- MRC Impairment may lead to intracellular ATP depletion.
- CoQ10 deficiency may lead to elevated ROS and ATP depletion
- CoQ10 deficiency accompanied by MRC inhibition.
- Elevated ROS may induce further damage to the MRC.
- To compensate there is an upregulation of mitochondrial biogenesis.

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Therapeutic potential of CoQ10 in the treatment of PD

- No consensus on the appropriate doses of CoQ10 to use in the treatment of disease or aging
- A plasma level of 5μM may be appropriate to induce biochemical/clinical benefit in patient, reference range 0.4- 2μM
- Main reported side effect is stomach upset- loss of appetite, nausea, vomiting, and diarrhea (gastro intestinal ; GI problems)

- In view of their superior absorption- gel /oil based and water soluble formulations of CoQ10 recommended in preference to tablets.

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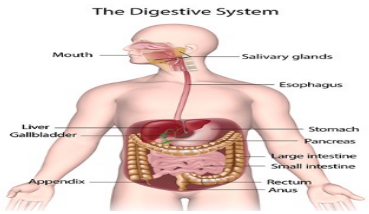
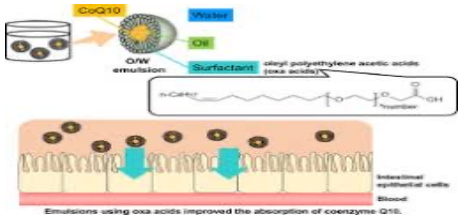
Therapeutic potential of CoQ10 in the treatment of PD





- CoQ10 is absorbed from the GI tract via a lipid carriers in the mucosal cells of the small intestine- initially into the lymph system, and then into the bloodstream.
- Following administration, CoQ10 takes approximately 6 hours to reach its maximal plasma concentration. Subsequently, a second plasma peak is often observed at about 24 hours.

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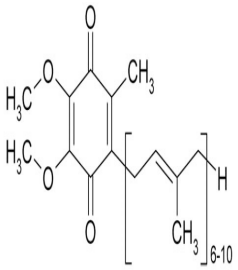
Therapeutic potential of CoQ10 in the treatment of PD



- In a Phase II clinical trial conducted by Schults and colleagues in 2002 oral CoQ10 supplementation (300–1200 mg/day) was found to *reduce the functional decline of patients with early-stage PD.*
- A subsequent Phase III clinical trial was conducted by Beal and colleagues in 2014 with PD patients receiving CoQ10 dosages of 1200 or 2400 mg/day *CoQ10 was well tolerated but showed no evidence of clinical benefit.*
- Contrasting findings of the clinical studies may reflect the broad range of sporadic PD patients used in the two clinical trials, with the heterogeneous patient populations contributing to their contradictory findings.

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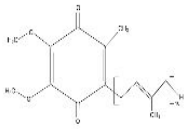
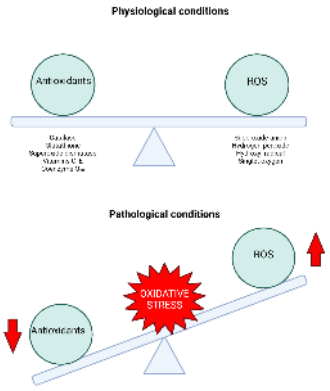
The potential neuroprotective effect of CoQ10 supplementation on oxidative stress neuronal cell model of Parkinson's disease



Aims: To assess the ability of CoQ10 (5 µM) supplementation (in its oxidised form) to ameliorate cellular and mitochondrial OS in neuronal cell model of PD.

Objectives:
- Investigate the potential antioxidative role of CoQ10 supplementation to prevent elevated ROS-induced OS in PD.



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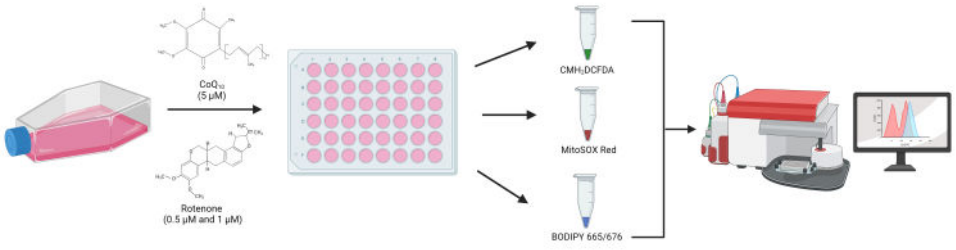
Effect of CoQ10 supplementation neuronal cell model of Parkinson's disease

- SH-SY5Y neuroblastoma human cell line
- Incubate with two concentrations of rotenone (0.5 µM and 1 µM) at 37 °C for 24 hours
- Followed by rotenone (0.5 µM and 1 µM) + CoQ10 (5 µM) at 37 °C for 24 hours
- MRC complex I deficiency maintained.



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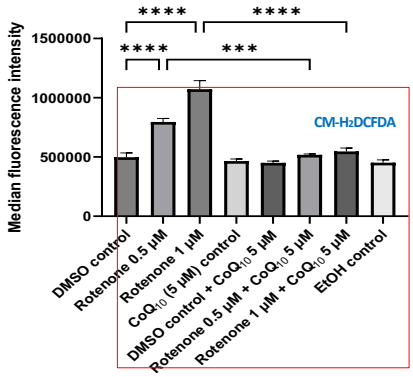
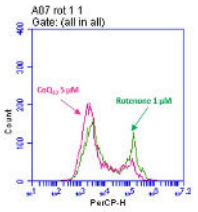
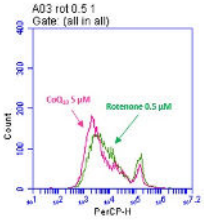
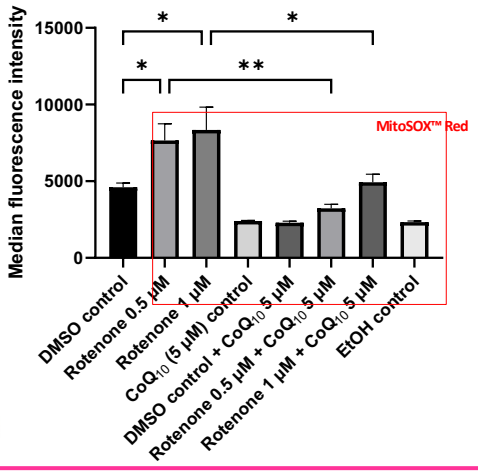
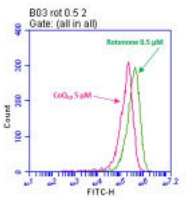
Effect of CoQ10 supplementation on neuronal cell model of Parkinson's disease

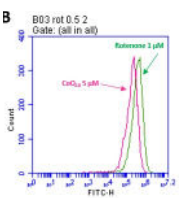












Fluorescence shifts detected using flow cytometry, showing the SH-SY5Y human neuroblastoma cell population significantly decreasing in fluorescence following CM-H_2DCFDA and MitoSOX™ Red staining post-CoQ_{10} (5 µM) treatment.

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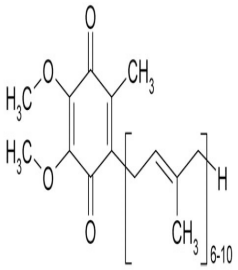
Therapeutic potential of CoQ10 in the treatment of PD



- At the cellular level CoQ10 supplementation appears to target OS decreasing the level of ROS to almost that of the control.
- Why the contrasting findings of clinical studies assessing the therapeutic efficacy of CoQ10 in the treatment of PD? Could the transport of CoQ10 to the brain have been a factor?
- Is CoQ10 able to cross the blood–brain barrier (BBB)?
- Whilst there is some evidence that CoQ10 may penetrate the BBB in some animal species, this has yet to be established in humans

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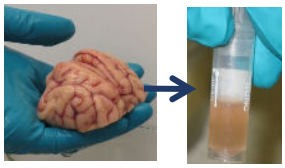
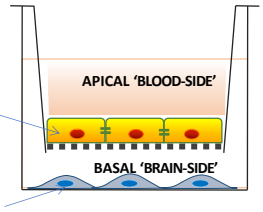
Can CoQ10 cross the BBB? In vitro models.

BBB Cell cultures: Porcine primary endothelia (PBEC) isolation and *in vitro* co-culture with primary astrocytes

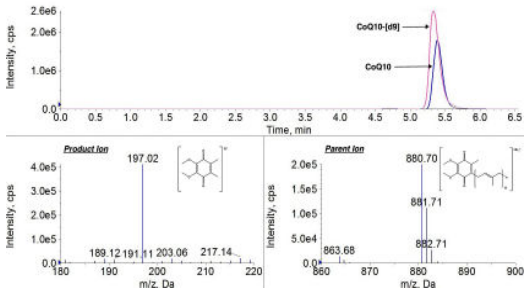




Blood-Brain Endothelial cells

Astrocytes

CoQ10 quantified using mass spectrometry (ESI LC-MS/MS)



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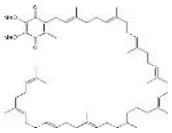
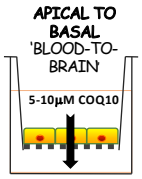
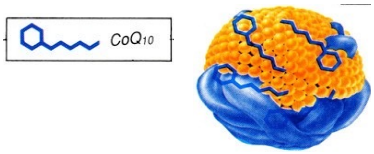
Coenzyme Q10



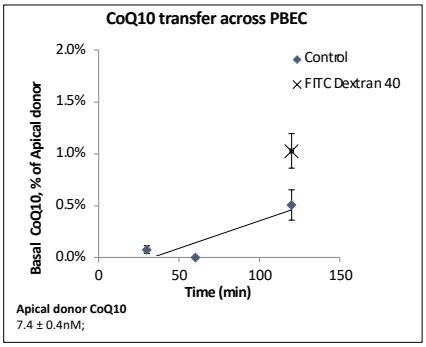
MW 863 g/mol
Experimental LogP 20
Oxidized form



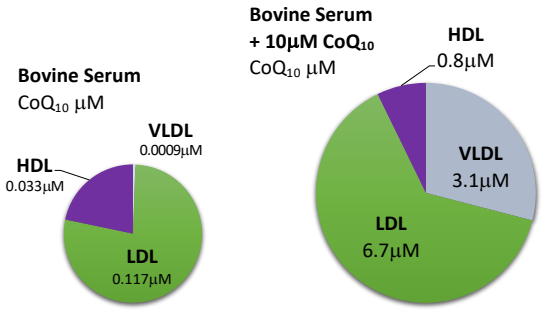
BUT: In vivo, CoQ10 is not free in plasma.
It adsorbs onto lipoproteins



CoQ10: No detectable transport until 2hr.
Smaller than FITC Dextran 40 BBB 'leak'



Incubate 10µM CoQ10 in bovine plasma 30 minutes.
Distribution of CoQ10 in Lipoprotein fractions



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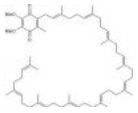
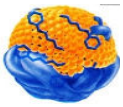
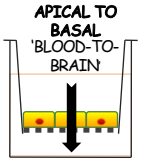
Co-Enzyme Q10
Control media

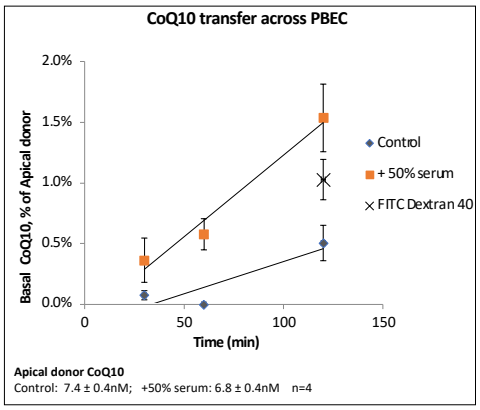


+50% serum





CoQ10 transport:
Detectable after pre-incubation in 50% serum medium



Is CoQ10 transport to the brain reliant on lipoprotein transport systems ?

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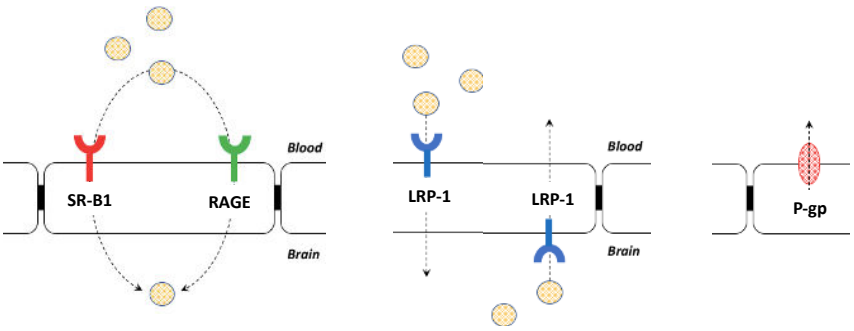
Selected BBB transporters:

Uptake | Bi-directional | Efflux



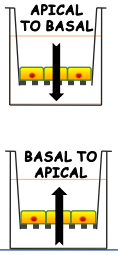
Inhibitors of lipoprotein/CoQ_{10} transport

Uptake 'Blood to Brain'

- **SR-B1** (Scavenger receptor B1) inhibitor BLT-1 10µM
- **RAGE** (Receptor Advanced Glycation End-products) inhibitor FPS-ZM1 1µM
- **LRP-1** (Low Density Lipoprotein related protein -1) inhibitor RAP 0.5µM

Removal 'Brain to Blood'

- **LRP-1** (Low Density Lipoprotein related protein -1) inhibitor RAP 0.5µM
- **ABCB1** efflux transporter (p-glycoprotein) inhibitor Verapamil 0.1mM



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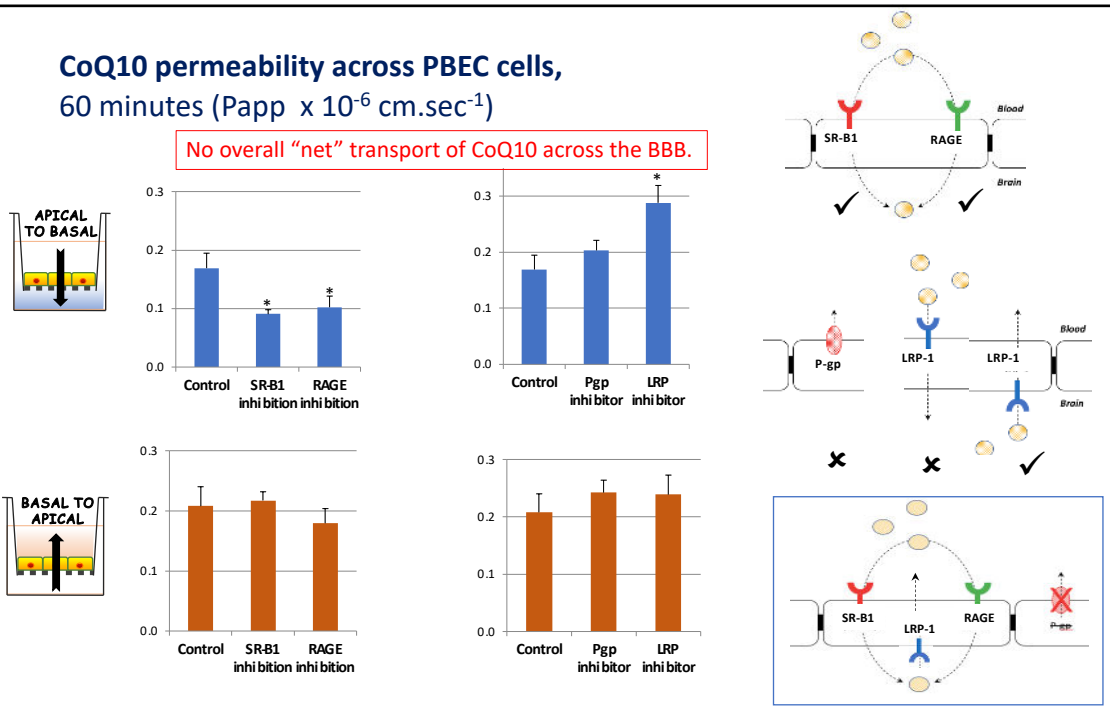
CoQ10 permeability across PBEC cells,
60 minutes (Papp x 10^{-6} cm.sec^{-1})

No overall "net" transport of CoQ10 across the BBB.



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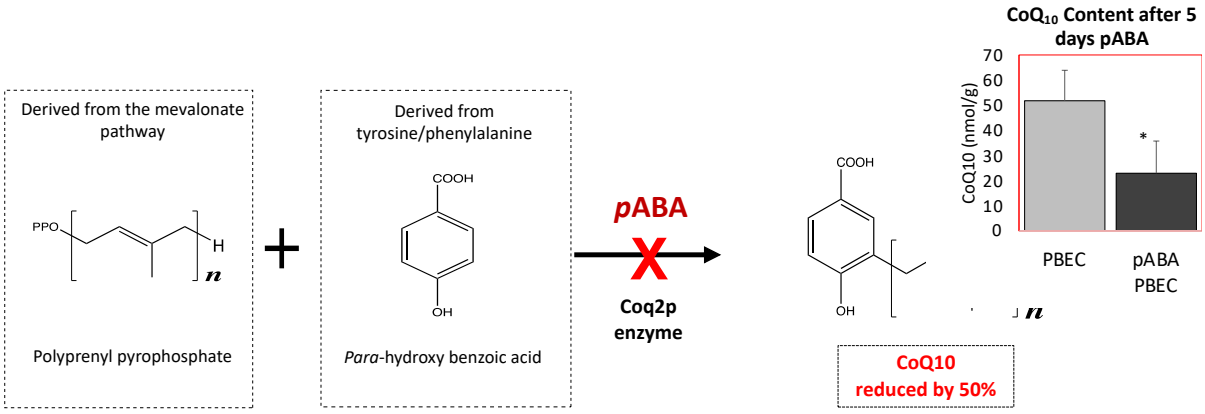
CoQ10 deficient BBB model

pABA (Para-aminobenzoic acid), inhibits an enzyme in the biosynthesis of CoQ10.
Five days culture with 1mM pABA, reduced CoQ_{10} 50% and MRC complex I, II-III activity by 70%.



Coq2p (polyprenyl-4-hydroxybenzoate transferase)

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