



Review

Role of mitochondria, oxidative stress and the response to antioxidants in myalgic encephalomyelitis/chronic fatigue syndrome: A possible approach to SARS-CoV-2 ‘long-haulers’?

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Received 9 October 2020

Available online 21 November 2020

Abstract

A significant number of SARS-CoV-2 (COVID-19) pandemic patients have developed chronic symptoms lasting weeks or months which are very similar to those described for myalgic encephalomyelitis/chronic fatigue syndrome. This study reviews the current literature and understanding of the role that mitochondria, oxidative stress and antioxidants may play in the understanding of the pathophysiology and treatment of chronic fatigue. It describes what is known about the dysfunctional pathways which can develop in mitochondria and their relationship to chronic fatigue. It also reviews what is known about oxidative stress and how this can be related to the pathophysiology of fatigue, as well as examining the potential for specific therapy directed at mitochondria for the treatment of chronic fatigue in the form of antioxidants. This study identifies areas which require urgent, further research in order to fully elucidate the clinical and therapeutic potential of these approaches.

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Keywords: Chronic fatigue syndrome; Mitochondria; Oxidative stress; Antioxidants; SARS-CoV-2

Introduction

A significant proportion of patients recovering from the current SARS-CoV-2 pandemic, termed ‘long-haulers’ or ‘Long COVID’, are reporting a broad range

of persistent and debilitating symptoms centred around fatigue but also including symptoms such as ‘brain fog’, pain, breathlessness, and dysrhythmias, that have extended several months into the post infection period.^{1,2} These symptoms are a characteristic of an already well-documented, potentially devastating and largely unexplained post-viral illness, myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS). This disease is already known to be associated with other viral infections such as Epstein Barr or glandular fever.³ ME/CFS is a disease of unknown pathophysiology affecting 0.2%–2.0% of the world’s population.^{4,5} It is characterised by severe post-

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Peer review under responsibility of Chinese Medical Association.



exertional fatigue that is not relieved by rest, and as well as pain, severe cognitive dysfunction, insomnia and/or lack of restful sleep, and sensitivity to light and sound, lasts for longer than six months.⁶ There is now growing belief that, with millions of SARS-CoV-2 infections worldwide, there will be a spike in ME/CFS, with some people's post viral fatigue developing into the a long term illness that is symptomatically the same as ME/CFS —the unknown question is how many will be affected?

Knowing how many ME/CFS patients there are is difficult: withdrawal from society is common,⁷ especially as one in four individuals can be bed- or housebound lifelong, and many more whilst in the acute phase.^{4,5} Demographic data collection is also hampered because there is no simple diagnostic test for this disease, (twenty published, differing clinical definitions exist).⁴ It is approximately two to six times more common in females than males,⁸ and affects all ages, socio-economic groups, ethnic groups and geographical locations.⁴ It is unknown, as yet, whether the 'long-haulers' from SARS-CoV-2 infections with ME/CFS like symptoms will share the same gender bias and other characteristics. Similar conditions to ME/CFS have been known for the last two centuries but not recognised as such.⁹ In 2019, the Senate of the United States passed a resolution acknowledging that ME/CFS was a serious disease that affected many people and in great need of more research.¹⁰ Spurred on by the SARS-CoV2 pandemic, research into ME/CFS may see such a resurgence. This would be a cruel irony for long-term ME/CFS sufferers if their condition, which has taken so long to be acknowledged as a serious debilitating disease, now gets ready acceptance through COVID-19.

The lack of a known aetiology or pathophysiology, the variability in the symptoms and the lack of outward physical signs of the illness have all contributed to the societal stigma of ME/CFS.¹¹ It has often been referred to as an 'invisible disease',^{12–15} as people may appear outwardly healthy and well, but be severely ill.^{11,15} Previous classification of ME/CFS as a psychiatric disorder has hindered the care of sufferers of the disease and their interactions with their physicians and the public for many years.^{15,16} It is hoped the SARS-CoV-2 'long-haulers' will not have to undergo these experiences before their illness is properly acknowledged. A 2016 survey found that 60% of affected individuals were not diagnosed for at least one year and this delay can worsen the psychosocial impacts of the disease.⁴ These diagnostic difficulties make it hard to know whether the syndrome is a single disease entity with a range of symptoms, rather like multiple sclerosis (MS),

or rather several different diseases with very similar and overlapping symptomatology. For example, Booth et al¹⁷ argues for two or more distinct pathophysiologicals that are grouped together as ME/CFS. ME/CFS can have a number of initiating triggers, the most common being a viral infection.

The focus of this review is the links between some of the dysfunctional pathways found in ME/CFS — specifically mitochondrial dysfunction and oxidative stress — and the possible therapeutic effects of antioxidants levels upon these pathways. Simple remedies like antioxidants that are readily available and that can control oxidative stress might prove helpful to improve the lives of ME/CFS patients, as well as those suffering from post-viral illness as a result of SARS-CoV2 infections before other therapies become available. Mitochondria are organelles that are present in every cell of the body and produce 90%–95% of the body's total energy. Mitochondrial respiration produces the energy-carrier adenosine triphosphate (ATP) which drives all the necessary chemical reactions in the body. Cells only contain a small steady state concentration of ATP molecules in cells which needs to be constantly re-generated because of energy demands. This is done by oxidative phosphorylation (OXPHOS) or by glycolysis.¹⁸ Maximal mitochondrial respiration increases with stress, and there can be up to a 100-fold increase in ATP consumption during vigorous activity compared with sleep. Mitochondria are also involved in a wide range of other important cellular actions including redox signalling and cell danger responses.^{19,20} Dysfunction in any of these functions may possibly account for clinical symptoms in ME/CFS,²⁰ however, mitochondrial bioenergetics is the main area of focus of this review, because dysfunction in mitochondrial respiration and metabolic changes have been found in ME/CFS,^{21–23} and these give a biologically plausible link to some of the symptoms seen in ME/CFS.²⁴ A recent review²⁵ has looked at mitochondrial changes in ME/CFS patients compared to healthy controls across 19 studies. They concluded that it was difficult to establish the role of mitochondria in the pathology of ME/CFS due to inconsistencies across the published studies. They argued for future studies to be well-designed, with patients diagnosed with the same ME/CFS clinical case definition and with analyses using the same methods. Despite this review, we argue that there is evidence of mitochondrial disturbances in ME/CFS and our review focuses on the potential of exploiting these for therapeutic approaches to alleviating both this complex disease and for SARS-CoV-2 chronically ill patients.

The search for peer-reviewed articles was conducted using Medline and Google Scholar up to March 2020. The following search terms were used: myalgic encephalomyelitis/chronic fatigue syndrome, coenzyme Q10 (CoQ10), MitoQ, bioenergetic health index (BHI), oxidative stress, mitochondrial dysfunction. Variations of these terms were used to ensure all possible papers were found. Inclusion criteria included the English language.

Mitochondrial energy pathways and dysfunction

A growing body of literature has investigated mitochondrial bioenergetics in ME/CFS patients, and is reporting evidence of mitochondrial dysfunction^{21,26,27} but with varying results.^{25,28} Since mitochondria produce most of the body's energy, they could contribute to ME/CFS symptoms of muscle pain, weakness and cognitive decline.²⁹ Mitochondrial dysfunction found with classical mitochondrial disease caused by genetic mutations, share symptoms of fatigue, muscle weakness, cognitive decline and a waxing and waning pattern such as seen in ME/CFS.²⁹

A range of different markers has been used to measure mitochondrial dysfunction in ME/CFS including the levels of certain mitochondrial proteins,³⁰ production of ATP^{17,27,31} and more recently, oxygen consumption of live plated cells using a new technology with the Seahorse Analyzer.^{21,22,32} The earliest evidence of mitochondrial dysfunction in ME/CFS was structural changes seen in the skeletal muscle cell mitochondria.³³ This study observed branching and fusion of cristae within the mitochondria, and variable shape and size in these patients. Lawson et al³⁴ also found condensation of cristae, but no difference in the shape and size of the mitochondria in peripheral blood mononuclear cells (PBMCs). Both studies concluded that the increased energy demand in ME/CFS caused this excess branching of cristae in mitochondria.

Dysfunctional energy producing pathways have been shown in ME/CFS.^{17,26,34} Booth et al¹⁷ and Lawson et al³⁴ each suggested compensatory enhancement of other pathways such as anaerobic glycolysis occurred in ME/CFS, to compensate for dysfunctional oxidative phosphorylation. An increase in glycolysis causes damage and contributes to other dysfunctions.¹⁷ In contrast, Tomas et al²¹ found no change in glycolysis compared to controls. However they did find an overall decreased OXPHOS. Armstrong et al²⁶ suggested glycolysis may be inhibited as

lactate levels were low, when investigating metabolic pathways.

Tomas et al²¹ found that OXPHOS in PBMCs of ME/CFS patients was significantly depressed when compared to controls, and Booth et al¹⁷ found decreased ATP production by OXPHOS. Missailidis et al²² determined that ATP synthesis, as a percentage of basal respiration, was decreased in ME/CFS compared to controls which suggested a defect in ATP synthase (complex V) in ME/CFS. They inferred this was causing compensatory increases in the rest of the electron transport chain (ETC) (Fig. 1), as they found increased levels of other complexes: this suggested mitochondria were unable to increase energy from baseline when required. Two studies^{21,35} found mitochondria had a lower reserve capacity for energy production in ME/CFS compared to healthy controls, and also suggested mitochondria in ME/CFS are unable to meet energy demands during exercise or acute stress, giving a potential reason for the severe post-exertional fatigue seen in patients.

Lawson et al³⁴ and Tomas et al³² found no differences in the levels of specific complexes I-IV of the ETC between ME/CFS and healthy controls, despite finding changes in OXPHOS as a whole in their previous paper.²¹ They suggested that any dysfunction, therefore, was not due to these complexes but instead to an external stimuli or other cause such as oxidative damage. Oxidative damage may be a contributor to the dysfunction seen in mitochondrial pathways.³⁶ Castro-

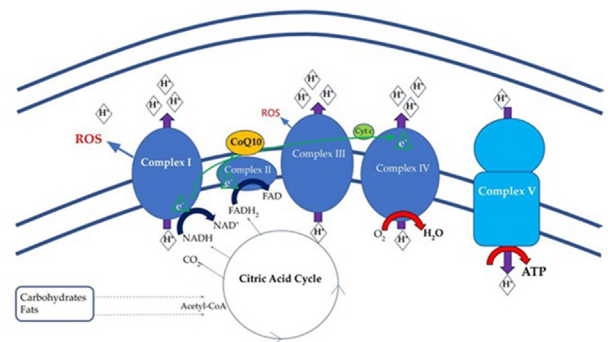


Fig. 1. Electron transport chain on the inner mitochondrial membrane. Electrons move from carriers NADH and FADH2 to oxygen, the final acceptor. CoQ10 (yellow) moves from Complex I or Complex II carrying electrons to Complex III. The electrons then are carried from Complex III to Complex IV by cytochrome C (labelled Cyt C, green). NADH: Reduced nicotinamide adenine dinucleotide; FADH2: Reduced flavin adenosine dinucleotide; ROS: Reactive oxygen species; CoQ10: coenzyme Q10. (Image source: Sweetman et al,⁸ with permission).

Marrero³⁷ reported higher lipid peroxidation as a cause of lowered ATP levels in ME/CFS compared to healthy controls. Morris and Maes³⁶ suggested reactive oxygen species and inflammation might be a cause of mitochondrial damage and dysfunction. Missailidis et al,²² however, suggested that the cause of dysfunction was a defect in the control pathway of ATP synthase and Vermeulen et al³⁵ suggested a decreased ability for ME/CFS to deliver oxygen to cells caused dysfunction and decreased ATP production. Lawson et al³⁴ described changes in the mitochondria indicating ME/CFS patients have an increased and unmet energy demand instead of overt mitochondrial dysfunction.

Referring again to Fig. 1, an analysis of the proteomes of a cohorts of ME/CFS patients and healthy controls by Sweetman et al⁸ showed a significant proportion of proteins with changed expression in the ME/CFS cohort, were involved in mitochondrial function, oxidative phosphorylation, ETC complexes (shown in blue) and redox regulation or reactive oxygen species. These changes would contribute to oxidative stress.

Oxidative stress

Oxidative stress is a condition of increased amounts of reactive oxygen/nitrogen species. It is an important pathological feature of many acute and chronic diseases as well as the normal aging process. Oxidative stress results from an imbalance between free radicals and antioxidants which causes an increase in free radicals.³⁸ Free radicals have an additional unpaired single electron, which is unstable and can cause damage to biological proteins and DNA.³⁹ Free radicals are also produced in normal physiological cell action and signalling.⁴⁰ Reactive oxygen species (ROS) are molecules which include free radicals and at least one oxygen atom, such as superoxide or hydrogen peroxide. Antioxidants are scavengers that remove free radicals and are necessary to prevent cellular damage.³⁹ Mitochondria are a major producer of ROS in the cell, which makes them very susceptible to damage by oxidative stress.⁴⁰ In the mitochondrial ETC, electrons en route to oxygen may be diverted in the conversion of oxygen into superoxide.⁴¹ In the mitochondria there are enzymes and coenzymes, such as vitamin E and CoQ10, that remove ROS to prevent damage. If there is damage or dysfunction in the ETC and/or in the ROS-removal system of mitochondria – for example with low levels of CoQ10 – ROS will increase causing further damage.⁴⁰

Oxidative stress in ME/CFS

Increased oxidative stress is important in causing and perpetuating a range of pathologies.⁴² Jammes et al⁴³ showed increased markers of oxidative stress in 38 ME/CFS participants and also showed these individuals had greater oxidative stress after exercise. Richards et al⁴⁴ also found an association between increased levels of oxidative stress and ME/CFS symptoms in 33 patients. Castro-Marrero et al³⁷ found increased signs of oxidative damage and decreased antioxidants (CoQ10) in mitochondria from patients with ME/CFS. Miwa and Fujita⁴⁵ found levels of oxidative stress were increased in the relapse state of ME/CFS. Oxidative stress found in ME/CFS could be contributing to mitochondrial dysfunction, inflammatory pathways, and brain dysfunction.³⁶

Suitable biomarkers of oxidative modifications of disease are oxidation products that are stable, can be measured, and which correlate with disease severity so that they can be used as diagnostic tools. Commonly used biomarkers of oxidative stress are: (a) a physiological ketoaldehyde produced from decomposition of unsaturated lipids that combines with free amino groups, mainly lysine of proteins; (b) isoprostanes, a family of prostaglandin F2a -like compounds that can be analysed in body fluids including urine and plasma; (c) glutathione disulphide/glutathione ratios; (d) protein carbonyls that are very stable in plasma samples stored at –80 °C degrees; and (e) 8-oxodeoxy guanosine from modified DNA. A number of specific analytical methods have been developed to analyse these oxidation products as an indicator of oxidative stress.⁴⁶

Mitochondrial therapies for ME/CFS

As more knowledge of the mitochondrial effects has been learnt, potential treatments are being targeted to improve mitochondrial health.^{47–55} Teitelbaum et al⁵⁰ has investigated the effect of D-ribose, the sugar moiety of ATP that stimulates energy production, on ME/CFS symptoms in an uncontrolled, open-label study. They found significant improvement in fatigue as measured by visual analogue scales. ME/CFS patients taking methylphenidate (Ritalin®) with vitamins and minerals which support mitochondrial function, reported some improvement in fatigue scores, although this was non-significant when compared to placebo.⁵¹ In an uncontrolled, open-label study, 45% of participants had reduced fatigue when treated with sodium

dichloroacetate, a pyruvate dehydrogenase/glycolysis inhibitor.⁵² These studies may be relevant to SARS-CoV-2 recovering patients.

CoQ10 and mitochondria

CoQ10 (also called ubiquinone) is a potential treatment under investigation. It is an important antioxidant in the mitochondria. CoQ10 is the only lipid-soluble antioxidant that is endogenously synthesised in humans.⁵⁶ It consists of a quinone head which can complete reduction–oxidation reactions and a long polyisoprenoid lipid tail making it extremely hydrophobic; this allows it to move freely within the phospholipid bilayer.⁵⁶ Ubiquinol (reduced CoQ10) acts as an antioxidant by reducing ROS, or by regenerating other antioxidants such as vitamin E.⁵⁷ After neutralising an oxygen radical, enzymes in the ETC can recycle and reduce CoQ10 back to ubiquinol.

A significant proportion of CoQ10 (50%) is found in the mitochondrial membranes,⁵⁸ but it is also present in all membranes of the body and in the plasma where it is carried in the lipoproteins low-density lipoprotein (LDL) and very low-density lipoprotein (VLDL).⁵⁹ CoQ10 is found in the highest concentrations in highly metabolic tissues such as the heart, liver and muscle.⁵⁸ Detection of CoQ10 in muscle cells is considered the gold standard for detecting defects in the synthesis of CoQ10.⁵⁷

CoQ10 levels vary amongst individuals. Tissue levels are controlled by endogenous synthesis,⁶⁰ while plasma levels can be influenced by a range of other factors, including diet,⁶¹ gender,^{62,63} hormones⁶⁴ and exercise.^{65,66} Vitetta et al⁶⁷ also suggested tissue breakdown, associated with aging, may increase plasma CoQ10 levels due to cell CoQ10 leaking into plasma. Niklowitz et al,⁶² however, measuring serum CoQ10 levels in 840 individuals, showed an inverse U-shaped curve with age, with middle-aged adults having the highest levels. Different studies have found widely varying ranges in healthy populations.⁶⁸

When a person is well, quantities of CoQ10 in tissues are thought to be sufficient due to the endogenous production.⁶⁹ Beal⁷⁰ suggested that the level of CoQ10 in membranes reaches a saturation level in young animals, so that supplementation with CoQ10 does not further increase brain tissue levels. CoQ10 levels did increase in the brain tissue of older individuals and it was suggested that supplements may be beneficial in humans with decreased synthesis such as the elderly or for those with primary CoQ10 deficiency or other

diseases.⁷¹ López-Lluch et al⁷² discussed that cells may only take up CoQ10 from plasma, if they are unable to produce it sufficiently. Therefore, CoQ10 supplementation may only produce a clinical effect if the disease is associated with low CoQ10 levels.

CoQ10's role is vital in the mitochondria. Genetic disorders which result in little to no CoQ10 have severe debilitating symptoms.^{56,73} A genetically-based failure of the synthesis of CoQ10 causes some forms of mitochondrial disease.⁷⁴ There is no reported genetic disorder caused by an excess CoQ10. Lowered levels of plasma CoQ10 have been reported in a range of diseases including Alzheimer's disease, diabetes, and 'low-energy syndromes', such as Prader–Willi syndrome, Friedrich's ataxia, cancers, as well as hereditary mitochondrial disorders.⁵²

Primary CoQ10 deficiency is defined as a deficiency due to mutations in the genes encoding CoQ10 synthesis. Primary CoQ10 deficient patients can have a range of phenotypes that affect highly metabolic tissues such as the brain, skeletal muscle and kidneys, resulting in nephrotic syndrome, cerebellar ataxia, myopathy and/or encephalopathy.⁵⁷ Supplemental CoQ10 can treat primary CoQ10 deficiency.⁷⁴

Secondary CoQ10 deficiency is caused by mutations in genes in other pathways such as those causing OXPHOS defects, which in turn produce low CoQ10 levels, or it can be associated with disease or drugs such as statins.^{56,74} Rodríguez-Aguilera et al⁷⁴ discusses that secondary CoQ10 deficiency could be caused by compensating for a dysfunctional OXPHOS, which can occur in ME/CFS.

CoQ10 and ME/CFS

There is reported evidence that CoQ10 levels are decreased in ME/CFS.^{37,75,76} There is not strong enough evidence, however, to call ME/CFS a secondary CoQ10 deficiency syndrome, as measurement of fibroblast CoQ10 levels is necessary to define secondary CoQ10 deficiency.^{55,77} Maes et al⁷⁶ studied 58 ME/CFS participants and 22 healthy controls, and measured CoQ10 levels in plasma and severity of symptoms using a 'fibrofatiigue' (FF) scale. They found many ME/CFS participants had CoQ10 concentrations lower than the lowest healthy control value (490 µg/L), and that the CoQ10 concentration had significant negative correlation with the FF scores. They concluded that CoQ10 had a role in the pathophysiology causing ME/CFS symptoms. Castro-Marrero et al³⁷ found significantly lower levels of CoQ10 in peripheral blood mononuclear cells (PBMCs) in 23

ME/CFS participants compared to 15 control participants. They also found significantly decreased ATP levels and increased lipid peroxidation and argued these indicated mitochondrial dysfunction in ME/CFS. Kurup and Kurup⁷⁵ found statistically significant lower levels of CoQ10 in plasma of 15 ME/CFS patients compared to 15 control participants. They suggested magnesium deficiency, low CoQ10 levels and dysfunction of ion transporter Na⁺/K⁺ + ATPase all contribute to mitochondrial dysfunction in ME/CFS. By contrast, Wood,⁷⁸ found no statistical differences between an ME/CFS cohort and healthy controls for CoQ10 in PBMCs or plasma.

CoQ10 has been shown to restore respiration and act as an antioxidant.⁷⁹ Oral supplementation of CoQ10 has been researched in low-CoQ10 conditions including mitochondrial disease,⁸⁰ neurodegenerative diseases⁸¹ and ME/CFS.^{47,48,54} Primary CoQ10 deficiency can be treated by supplementation with CoQ10, however there is a mixed response to supplementation for those affected by secondary CoQ10 deficiency.⁷⁴ Fukuda et al⁵⁴ found that supplementation with CoQ10 significantly increased ME/CFS patients plasma levels of CoQ10. There was a significant correlation between increasing CoQ10 concentration and decreasing oxidative stress and fatigue.

Castro-Marrero et al^{47,48} assessed clinical outcomes after supplementation with reduced nicotinamide adenine dinucleotide (NADH) and CoQ10 in ME/CFS. Castro-Marrero et al⁴⁷ also claimed a direct improvement in mitochondrial function by finding a statistically significant increase in ATP levels and NAD⁺/NADH ratio in the intervention group. It is not clear if positive effects are due to CoQ10 alone or due to the combination of additional NADH, especially as treatment with NADH or CoQ10 alone had shown no changes in fatigue scores in previous studies.^{53,54}

A systematic review⁸² on sixteen studies of the effects of CoQ10 supplementation on fatigue for both healthy and cohorts with disorders, concluded that CoQ10 was better at decreasing fatigue in some disorders such as fibromyalgia and statin-related fatigue rather than ME/CFS. Mizuno et al⁸³ explored anti-fatigue effects of CoQ10 in healthy individuals and found that there was a non-significant trend for decreased perception of fatigue following exercise when CoQ10 was taken. They did find significant improvement in physical testing in CoQ10-supplemented groups compared to control groups, and suggested it was due to its antioxidant or OXPHOS roles. However, Orlando et al⁶⁵ found taking exogenous CoQ10 did not improve physical performance or

markers of muscular damage despite CoQ10 being depleted post-exercise in healthy individuals. These results of these studies are confusing and need clarification.

Bioavailability may account for this confusion (at least in part). CoQ10 has low bioavailability, due in part to its low water solubility and high molecular mass ($M = 863$ g/mol), which may explain why supplement studies have not always been consistent in producing clinical effects. It is also better absorbed if taken with a fatty meal, like other lipid-soluble supplements such as vitamin E.⁵⁸ Langsjoen and Langsjoen⁸⁴ found that ubiquinol had a greater bioavailability than ubiquinone in healthy subjects. Vitetta et al⁶⁷ also showed a non-significant trend for greater plasma CoQ10 levels at 2 hours after ubiquinol capsule supplementation compared to ubiquinone capsules in healthy individuals. Therefore, when assessing whether CoQ10 supplements might be beneficial for ME/CFS, the oxidative state of CoQ10 is important as well as the mode of administration.

Another important aspect to consider is the uptake of CoQ10 by tissues from plasma supplementation. In many clinical trials the extent to which CoQ10 enters cells is unknown. It has been found in rodent models that CoQ10 supplementation can increase tissue levels in the liver and spleen, but much higher doses are needed to increase levels in brain and muscle tissue.⁵⁸ Niklowitz et al⁸⁵ found that supplementing with CoQ10 increased levels in white blood cells and platelets but not in red blood cells. This low uptake in highly metabolic tissues may be a limitation of CoQ10 supplementation, as these are the tissues most influenced by CoQ10 deficiency.⁵⁶ Knowing how the molecule distributes across body tissues may be critical for CoQ10 to be used as an effective treatment for ME/CFS.

Mitoquinol mesylate (MitoQ ®)

A CoQ10 analogue, mitoquinol mesylate (MitoQ^{®86}), developed originally as a tool to target molecules to mitochondria,⁸⁷ is a powerful antioxidant and may be helpful in ME/CFS.^{78,87} It is now sold commercially as a supplement to improve health, energy levels and promote healthy aging. MitoQ is known 'to manipulate mitochondrial ubiquinone content *in vitro*.'⁸⁷ It has better bioavailability than CoQ10 due to its higher water-solubility. MitoQ consists of a CoQ10 molecule covalently attached to a lipophilic triphenylphosphonium (TPP⁺) cation. The positive charge allows MitoQ to accumulate in the negatively charged mitochondria⁸⁸ at a

concentration 50–1000 times higher than CoQ10.⁸⁷ This higher accumulation in the mitochondria and higher bioavailability accounts for its much lower recommended daily supplemental dosage of 10–20 mg compared to 200 mg for CoQ10. Thus, MitoQ may be a better candidate for mitochondrial therapy compared to CoQ10, as it may overcome the bioavailability and pharmacodynamic challenges of CoQ10 described above.

In the mitochondria, MitoQ is reduced by complex II of the ETC to form its reduced or ubiquinol state, becoming an antioxidant.^{79,87,88} Unlike CoQ10, it cannot be easily reduced by complex I or oxidised by complex III because the TPP + tail stops effective binding to these complexes. MitoQ does not restore respiration in the electron transport chain/OXPHOS as CoQ10 does, and is therefore not consumed by this process, allowing higher concentrations of its reduced state to persist within the mitochondria.⁸⁹

Kelso et al⁸⁷ found that MitoQ performed better than non-targeted (non-complex II-specific) antioxidants in the mitochondria, and prevented apoptotic cell death caused by hydrogen peroxide. MitoQ stopped mitochondrial dysfunction caused by oxidative stress. Park et al⁹⁰ found reduced reactive oxygen species in MitoQ-treated endothelial cells, possibly associated with decreased breakdown of the critical mitochondrial antioxidant enzyme, manganese-catalysed superoxide dismutase (SOD). Animal and human studies have been performed with MitoQ as a supplement.⁹¹ Individuals with chronic Hepatitis C virus showed positive benefits on liver function by decreasing alanine transaminase.⁹² A clinical trial found that MitoQ improved vascular function of healthy older adults, by showing increased brachial artery dilation, suggesting it may reduce the risk of cardiovascular disease.⁹³ Research investigating the effects on Parkinson's disease was, in contrast, inconclusive.⁹⁴

Johnson and Grant⁵⁵ performed a randomised controlled trial of MitoQ versus placebo in participants with ME/CFS conducted online via a website. Fifty one participants with ME/CFS were sent either MitoQ or placebo in the mail. No statistically significant improvement occurred. There were several limitations and potential biases to this study including a lack of ability to confirm the diagnosis, the usage of self-reported data and an inability to confirm compliance with the study. By contrast, another group of participants who took MitoQ without a matching placebo group, by purchasing the supplement themselves, found significant results and a reduction in clinical symptoms. So far, this online study is the only published study investigating MitoQ for ME/CFS.

Bioenergetic health index

The Bioenergetic Health Index (BHI) is an emerging measure in biochemistry that has been referred to as the ‘new BMI’ by the American Physiological Society.⁹⁵ It is a representation of mitochondrial health derived from calculating the ‘spare respiratory capacity’ multiplied by the ‘ATP production of mitochondria under stress’, divided by the ‘measured proton leak’ multiplied by the ‘non-mitochondrial respiration’ (glycolysis). Chacko et al⁹⁶ discussed the use of BHI as a marker of disease progression in particular for areas such as cancer, cardio-metabolic syndromes and neurodegenerative diseases, which are now being found to be affected by mitochondrial dysfunction.^{97,98} BHI is sensitive to oxidative stress and therefore may reflect changes in oxidative stress in these diseases.⁹⁶ BHI may help predict who is more susceptible to these diseases and who may benefit from emerging therapies targeting mitochondrial health. For example, post-cardiac surgery patients with the larger decreases in BHI values measured in leucocytes collected from their pericardial fluid were more likely to have cardiac complications (atrial fibrillation), than those with less significant changes in BHI values.^{99–104} As mitochondrial dysfunction and oxidative damage have been implicated in ME/CFS,^{28,29} BHI could be a potential biomarker for revealing how well the mitochondria are functioning over time and whether mitochondrial therapies such as MitoQ could be useful in ME/CFS. Only three analyses of BHI with ME/CFS patients have been completed with contradictory results.^{21,22,32}

Key papers regarding mitochondrial functions, oxidative stress and supplementation trials with ME/CFS patients are summarised in [Table 1](#).

Can antioxidant therapy help the ‘long haulers’ with SARS–CoV-2 infection?

The relatively high proportion of people chronically infected with SARS-CoV-2 (‘the long haulers’), who do not make a straight-forward recovery in the post viral period of their illness, almost certainly reflects damage done by the host response to the initial infection. A severe body response such as a cytokine storm can give rise to oxidative and inflammatory damage and generalised oxidative stress, and this suggests that the antioxidant therapies discussed in this review might be beneficial. Antioxidant therapy is known to improve the levels of the abundant natural antioxidant, glutathione (which is important for redox

Table 1
Key papers for ME/CFS patient studies.

Activity	Reference	Finding	Therapeutic Investigated	Cell Type/Tissue Investigated
Mitochondrial metabolic pathways	Fluge et al 2016 ¹⁰⁰	Impaired pyruvate dehydrogenase, consistent with inadequate ATP production, excessive lactate on exertion	NA	PBMCs
Metabolic response to exercise	Brown et al 2015 ¹⁰¹	Impaired AMP kinase activation, glucose uptake	NA	Skeletal muscle cells
Cellular metabolism	Naviaux et al 2016 ²³	Reduced metabolites in 20 biochemical pathways consistent with hypometabolic syndrome	NA	Plasma
Mitochondrial metabolism	Comhaire et al 2018 ⁵²	Reduction in fatigue severity consistent with existing mitochondrial hypometabolism and impaired pyruvate dehydrogenase	Sodium Dichloroacetate (Oral administration)	NA
Mitochondrial metabolism	Forsyth et al 1999 ⁵³	31% of patients had a positive reaction to NADH	NADH (Oral administration ENADA)	NA
Cellular energy synthesis	Teitelbaum et al 2006 ⁵⁰	Improvement in energy, sleep, mental clarity, pain intensity and well-being	D-ribose (Oral administration)	NA
Cellular stress, Inflammation	Sweetman et al 2019 ¹⁰²	Transcriptome -changes in stress, inflammation pathways, metabolic regulation, mitochondrial function, and circadian clock.	NA	PBMCs
Metabolic, Immune and Neurological	Helliwell et al 2020 ¹⁰³	Changes in DNA methylation indicate abnormal immune, neurological and metabolic functions	NA	PBMCs
Oxidative stress	Jammes et al 2005 ¹⁰⁴	Incremental exercise resulted in oxidative stress with alterations in muscle membrane	NA	Blood samples
Mitochondrial stress	Tomas et al 2017 ²¹	Lower maximal respiration indicating inability to compensate for stress	NA	PBMCs
Mitochondrial ATP production	Lawson et al 2016 ³⁴	Increased cristae density in patients. Increased ATP from non-mitochondrial sources	NA	PBMCs
Mitochondrial ATP production	Missailidis et al 2020 ²²	Complex V inefficiency in ATP production	NA	Immortalised lymphocytes
Mitochondrial complex activity	Tomas et al 2019 ³²	No observed differences in mitochondrial complex activity	NA	PBMCs, skeletal muscle cells
Mitochondrial Complexes, ATP production and oxidative stress	Sweetman et al 2020 ⁸	Disturbances in proteins of (a) mitochondrial complexes I & V, (b) regulating reactive oxygen species, and in mitochondrial pathways	NA	PBMCs
CoQ10	Maes et al 2009 ⁷⁶	Deficiency in CoQ10 in patient group correlating with symptom severity	CoQ10	Plasma
CoQ10	Castro-Marrero et al 2015 ⁴⁸	Improvement in fatigue levels and biochemical parameters (NAD+, CoQ10, ATP, citrate synthase and lipoperoxides)	CoQ10 (Oral supplementation)	
CoQ10/MitoQ	Wood 2020 ⁷⁸	An association of CoQ10 levels with mitochondrial function, enhanced by supplementation of cells with exogenous COQ10. Supplementation of MitoQ orally improved bioenergetic profiles of a subject.	CoQ10 MitoQ (oral supplementation)	PBMCs,
*MitoQ	Kelso et al 2001 ⁸⁷	MitoQ protects mitochondria from hydrogen peroxide-induced apoptosis	MitoQ	Human osteosarcoma cells
*MitoQ	James et al 2005 ⁷⁹	MitoQ10 was an effective antioxidant against lipid peroxidation, peroxynitrite and superoxide.	MitoQ	Rat heart mitochondria

* Non-patient studies showing MitoQ is a powerful antioxidant in mitochondria. BMC: Peripheral blood mononuclear cell; NA: Not applicable; NADH: Reduced nicotinamide adenine dinucleotide; CoQ10: Coenzyme Q10.

balance), and to strengthen the immune response.¹⁰⁵ Physiological changes in SARS-CoV-2 that enhance the production of reactive oxygen species could be ameliorated by free radical scavengers.¹⁰⁶ Oxidative stress and ongoing pathogenesis in SARS-CoV-2 are almost certainly linked.¹⁰⁷ Indeed Wu¹⁰⁸ believes the cytokine storm and free radical storm should be regarded as the actual pathogens.

These observations suggest antioxidant therapies should be trialled in patients struggling to recover after SARS-CoV-2. N-acetylcysteine is one therapeutic agent that has been suggested as an effective method of improving redox status, especially when under oxidative stress. Earlier clinical trials have used this compound to replenish glutathione stores and increase the proliferative response of immune T cells,¹⁰⁹ and indeed a new clinical trial to investigate vitamin C infusion for the treatment of severe SARS-CoV-2 infected pneumonia has already begun in Wuhan, China.¹¹⁰ Ouyang and Gong¹¹¹ hypothesize that MitoQ could act as a potential treatment for SARS-CoV-2 in the acute phase. Recent evidence indicates that mitochondrial dysfunction participates in the development of the illness and may be responsible for the ongoing abnormal immune response. On the basis of the research done with ME/CFS patients, we propose that MitoQ may alleviate the effects of the cytokine storm and restore the function of exhausted T cells through improving mitochondrial function, in ‘long haul’ or chronically ill SARS-CoV-2 patients as well.

Conclusion

Research into mitochondrial function of ME/CFS has been increasing in recent years and appears to hold potential for better understanding this enigmatic disease that may be increasing its prevalence as a result of the SARS-CoV-2 pandemic. So far, however, the results have proved somewhat contradictory. There are several reasons for this. An accurate diagnosis for ME/CFS is difficult in the absence a molecular biomarker diagnostic test, individuals in the ME/CFS group are often heterogeneous and have differing disease severity. There are multiple clinical case definitions that are being used for diagnosis. A vicious circle exists: without a biochemical diagnostic test it is difficult to be certain which patients have ME/CFS and not another fatigue illness with overlapping symptoms, but determining a valid test requires identifying an homogenous group of test subjects. If ME/CFS is indeed a state of CoQ10 deficiency — particularly in high energy-demand organs like the brain — then an

effective way of assessing CoQ10 is needed. This could then be a cellular biomarker that is monitored after oral supplementation in treatment of ME/CFS. Changes in mitochondrial function and markers of oxidative stress may be the biological component most impacted upon by CoQ10 supplementation, due to CoQ10's vital role in the electron transport chain. There is also clear need to establish whether MitoQ does indeed improve mitochondrial function and oxidative stress and lessens symptoms in both ME/CFS and chronic symptoms from SARS-CoV-19. There is a biologically plausible mechanism for expected improvement with MitoQ's superior bioavailability to cells and to mitochondria within them, and by how it can restore oxidative balance and therefore improve mitochondrial function. A respiratory index, like the BHI, also has the potential to predict and monitor pathophysiology in both ME/CFS and ‘long-haulers’ from SARS-CoV-2. Further research into ME/CFS and SARS-CoV-2, two perplexing and seriously debilitating diseases, is urgently required.

Funding

This study was support by a grant from Otago Medical School Scholarship for Ms Wood.

Conflicts of interest

None.

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Edited by Yi Cui