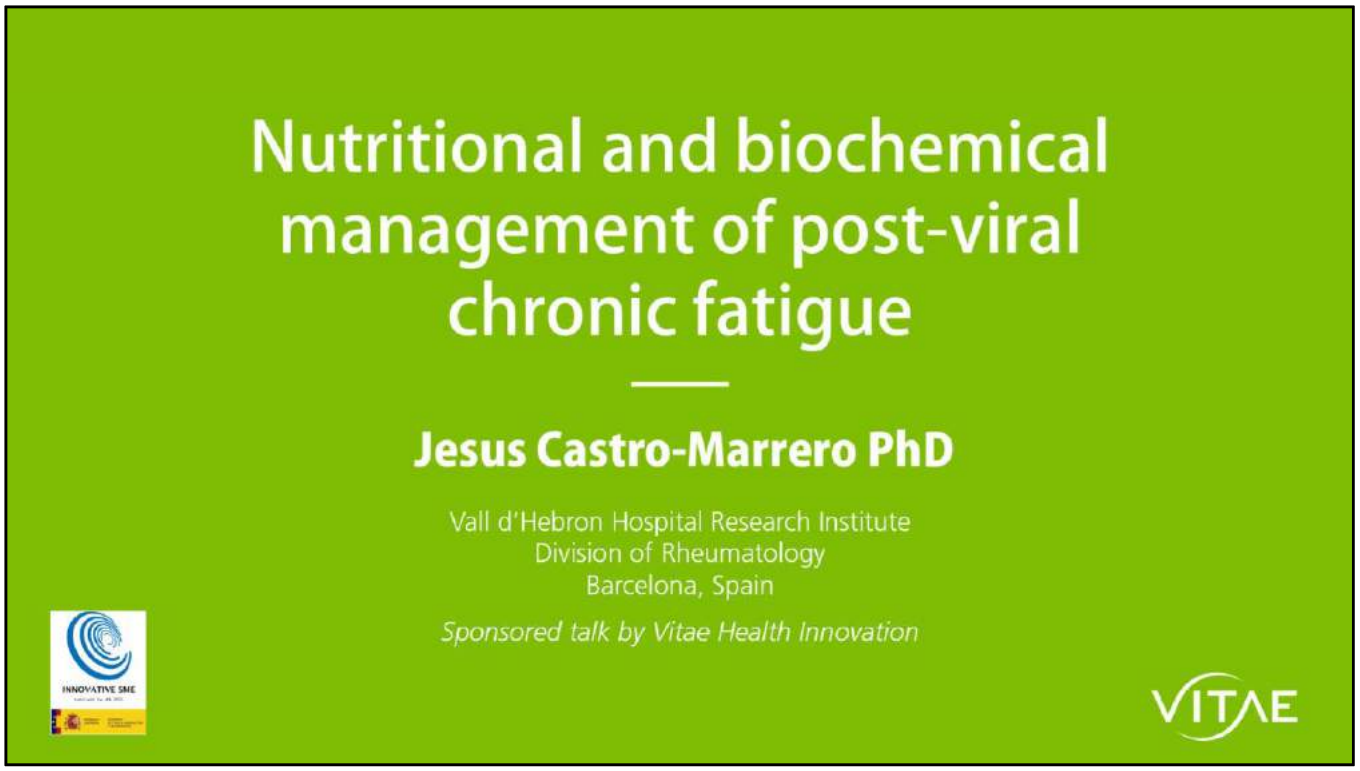


Jesús Castro, senior researcher at the Research Institute of the Vall d'Hebron University Hospital in Barcelona, Spain.

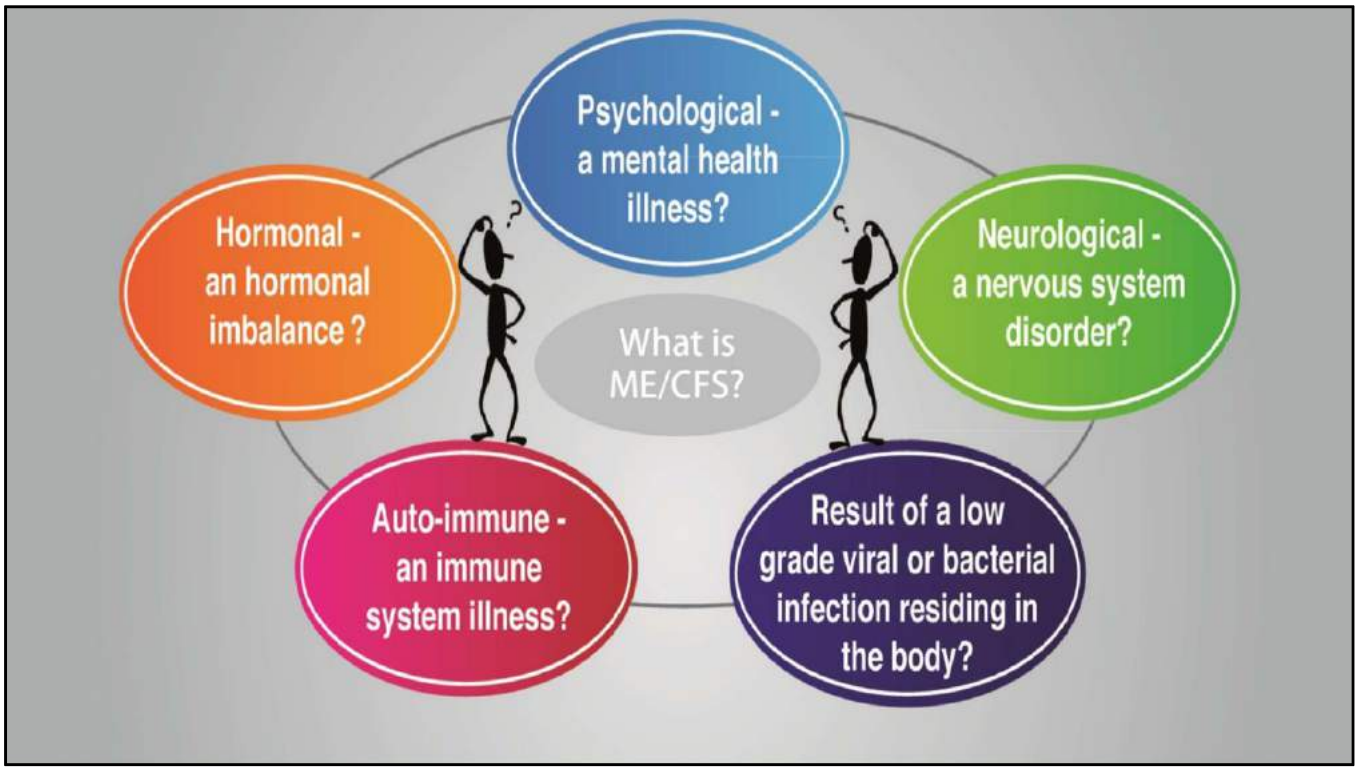
Today we will talk about the update on non-pharmacological treatments - nutritional supplements as a therapeutic approach in individuals with ME/CFS. We will also cover the management of post-viral chronic fatigue.

In addition, we will provide you with the latest results on the effect of oral supplementation of CoQ10 plus NADH and chronic fatigue-related symptoms in people with ME/CFS.

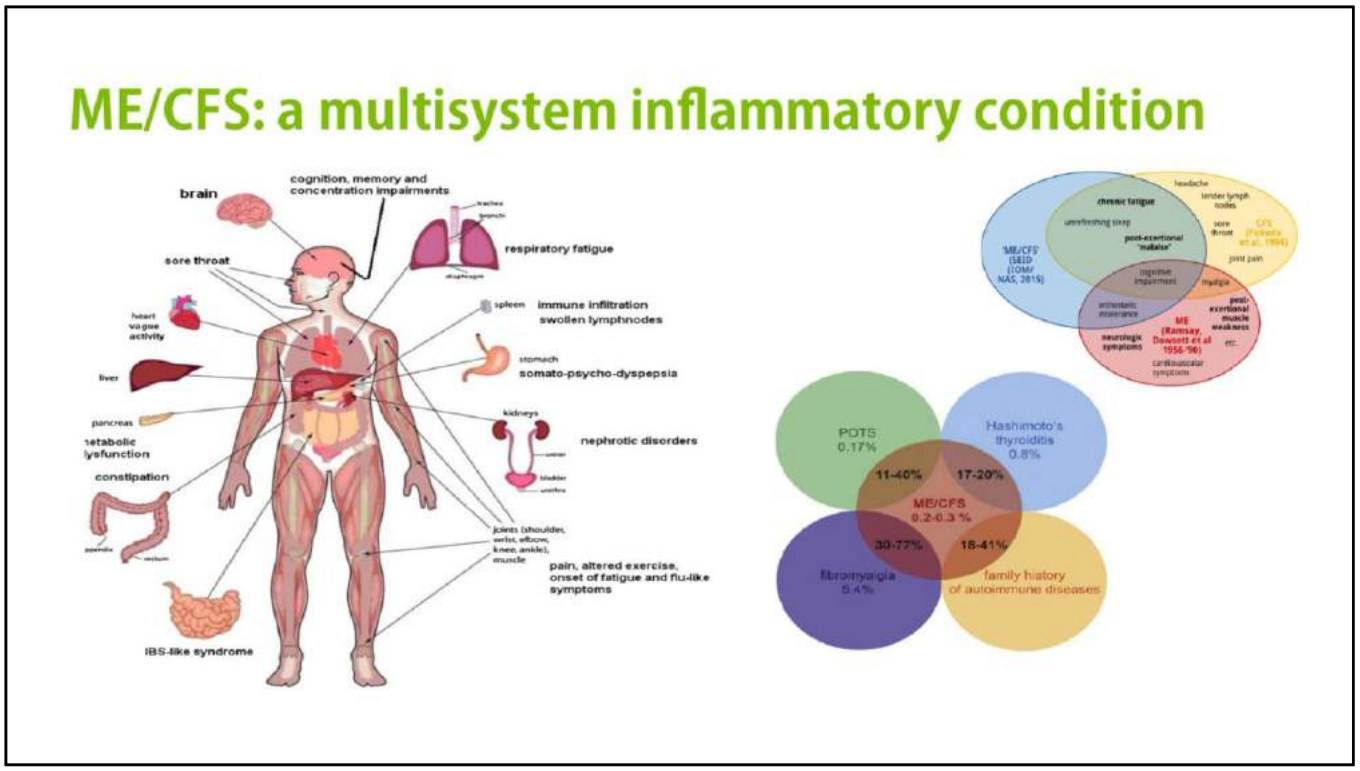
info@vitaeinternational.com
www.vitaeinternational.com

Roadmap

- Definition, etiopathology, case criteria and diagnostic biomarkers of post-viral fatigue syndrome (PVFS)
- Post-viral fatigue syndrome and COVID-19
- Challenges and controversies in the treatment and management of PVFS
- CoQ10 and NADH supplementation in ME/CFS as PVFS: findings from the Spain ReConnect study
- Take-home messages and future directions



Myalgic Encephalomyelitis/Chronic Fatigue Syndrome is a neuroimmune disorder characterized by extreme fatigue that can't be explained by any underlying medical condition. The post-exertional malaise (PEM) as hallmark symptom may worsen with physical or mental activity, but doesn't improve with rest. The cause of ME/CFS is unknown, although there are many theories — ranging from viral infections, immune dysfunction, hormonal imbalance, neurological impairments to psychological stress. Some experts believe ME/CFS might be triggered by a combination of these factors. In many cases, onset is linked to a viral infection.



ME/CFS is a frequent and severe chronic disease drastically impairing life quality. The underlying pathomechanism is incompletely understood yet but there is convincing evidence that in at least a subset of ME/CFS patients has an autoimmune etiology. Immune dysregulation in ME/CFS has been frequently described including changes in cytokine profiles and immunoglobulin levels, T- and B-cell phenotype and a decrease of NK cell cytotoxicity. Moreover, autoantibodies against various antigens including neurotransmitter receptors have been recently identified in ME/CFS individuals by several groups.

Furthermore, recent studies have provided evidence for severe metabolic disturbances presumably mediated by serum autoantibodies in ME/CFS. Therefore, further efforts are required to delineate the role of autoantibodies inthe onset and pathomechanisms of ME/CFS in order to better understand andproperly treat ME/CFS.


Post-exertional malaise


Cognitive/memory impairments ("brain fog")


Non-refreshing sleep


Orthostatic intolerance

The 2015 IOM diagnostic criteria for ME/CFS in adults and children state that **three symptoms and at least one of two additional manifestations are required** for diagnosis. The three required symptoms are:

1. **A substantial reduction or impairment in the ability to engage in pre- illness levels of activity**
2. **Post-exertional malaise (PEM)** – worsening of symptoms after physical, mental or emotional exertion that often puts the patient in relapse that may last days, weeks, or even longer. The symptoms typically get worse 12 to 48 hours after the activity or exposure.
3. **Unrefreshing sleep** – ME/CFS patients may not feel better or less tired even after a full night of sleep despite the absence of specific objective sleep alterations.

At least one of the following **two additional manifestations** must be present:

1. Cognitive impairment (aka brain fog) – patients have problems with thinking, memory, executive function, and information processing, as well as attention deficit and impaired psychomotor functions.

2. Orthostatic intolerance – patients develop a worsening of symptoms upon assuming and maintaining upright posture as measured by objective heart rate and blood pressure abnormalities during standing, bedside orthostatic vital signs,or head-up tilt testing. Orthostatic intolerance is often the most bothersome manifestation of ME/CFS among children/adolescents.

Many ME/CFS patients will also have, or appear to have, other medical problemsor related diagnoses (comorbid conditions). **Fibromyalgia** is common. It occurs in a large percentage of ME/CFS patients between onset and the second year, and some researchers suggest fibromyalgia and ME/CFS are conditions related.

Many ME/CFS sufferers also experience symptoms of IBS, temporomandibular joint pain, headache including migraines, and other formsof myalgia. ME/CFS is significantly more common in women with endometriosis compared with women in the general population.

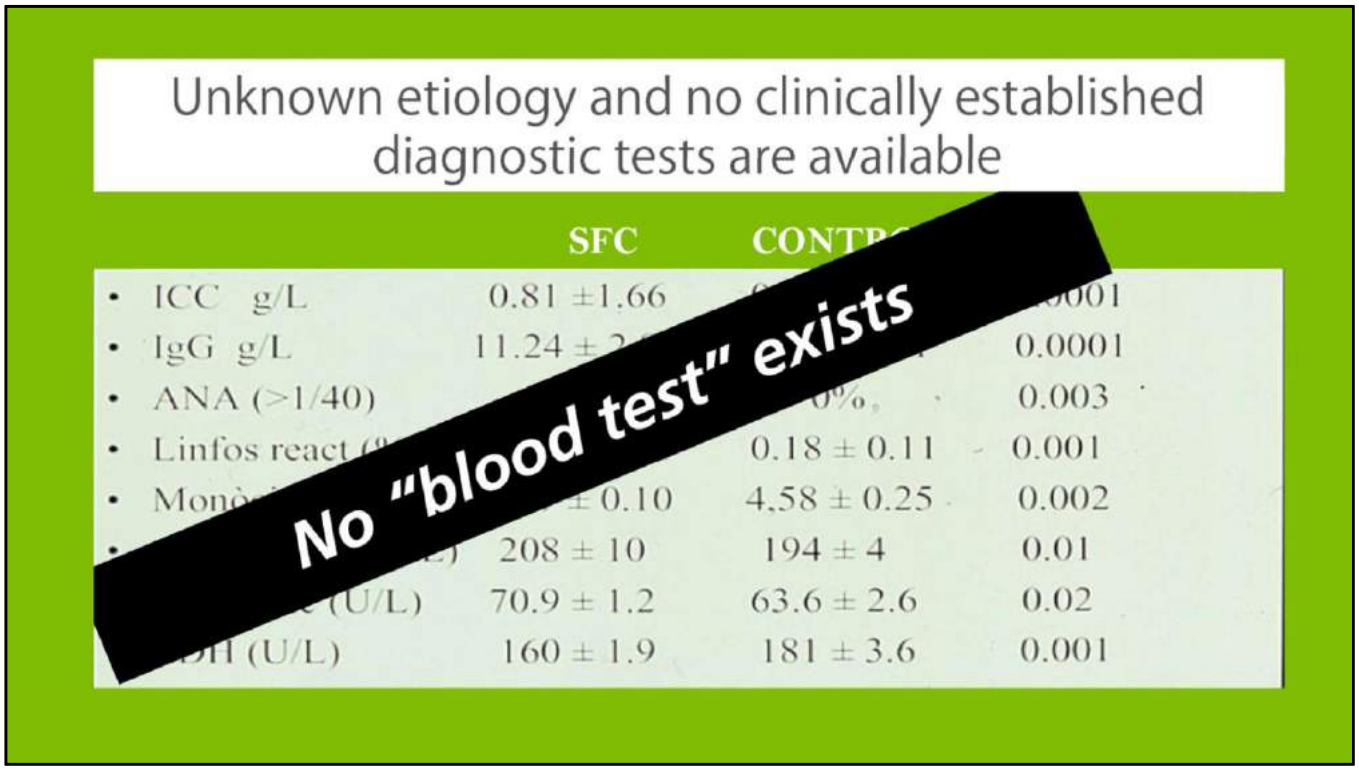
How could post-exertional malaise be measured in ME/CFS?

- 2-day consecutive CPET → most objective way to detect post-exertional malaise
- Diagnostic test: ME/CFS patient exercising on an exercise machine, while monitoring their respiration, especially oxygen consumption
- Abnormal results of ME/CFS patients on the 2-day CPET reflect post-exertional malaise
- 2-day CPET protocol can be used as an objective indicator that physical exertion decreases subsequent function in ME/CFS patients

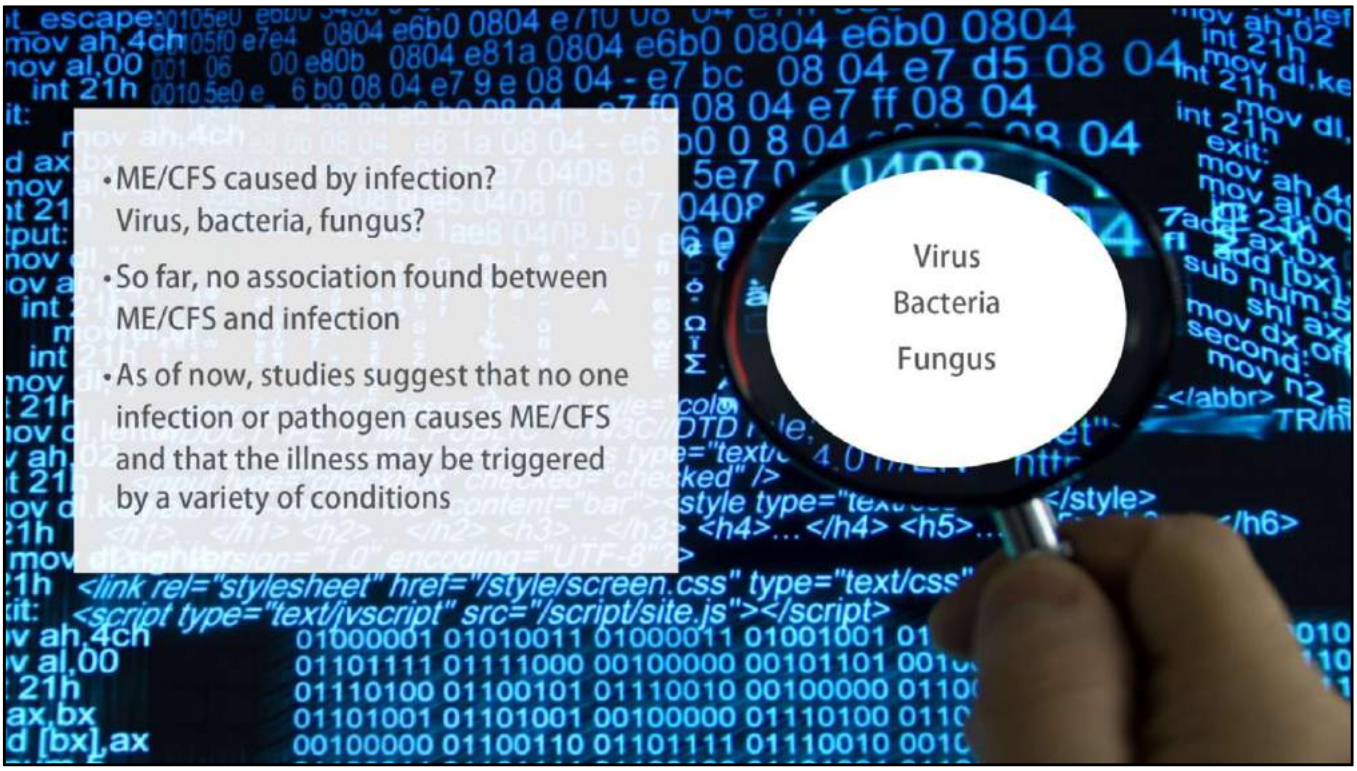
Previous evidence has shown that 2-day consecutive CPET is thought to be the most objective way to detect **post-exertional malaise**. This diagnostic test involves testing an ME/CFS patient exercising on an **exercise** machine, while monitoring their respiration, especially oxygen consumption.

According to researchers in ME/CFS, the abnormal results of ME/CFS patients on the 2-day CPET reflect post-exertional malaise, a marked symptom exacerbation after exercise thought to be characteristic of this condition.

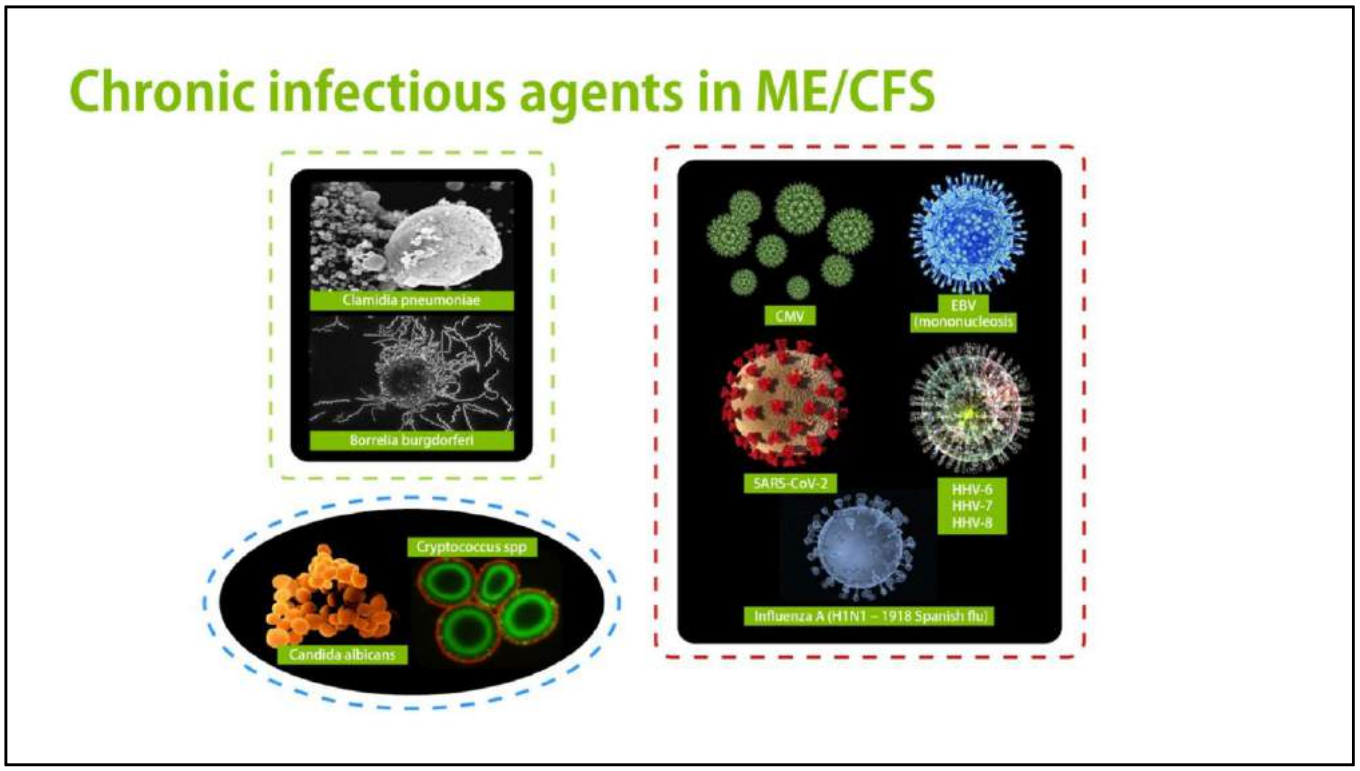
The 2015 report by National Academy of medicine indicated that the 2-day CPET protocol can be used as an objective indicator that physical exertion decreases subsequent function in ME/CFS patients. The 2-day CPET protocol however is not required inmaking the diagnosis of ME/CFS. Some have expressed concern that exercise tests may significantly worsen the condition of ME/CFS patients.



Currently, there are no highly-specific gold standard tests available, or any FDA-approved drugs for treatment.

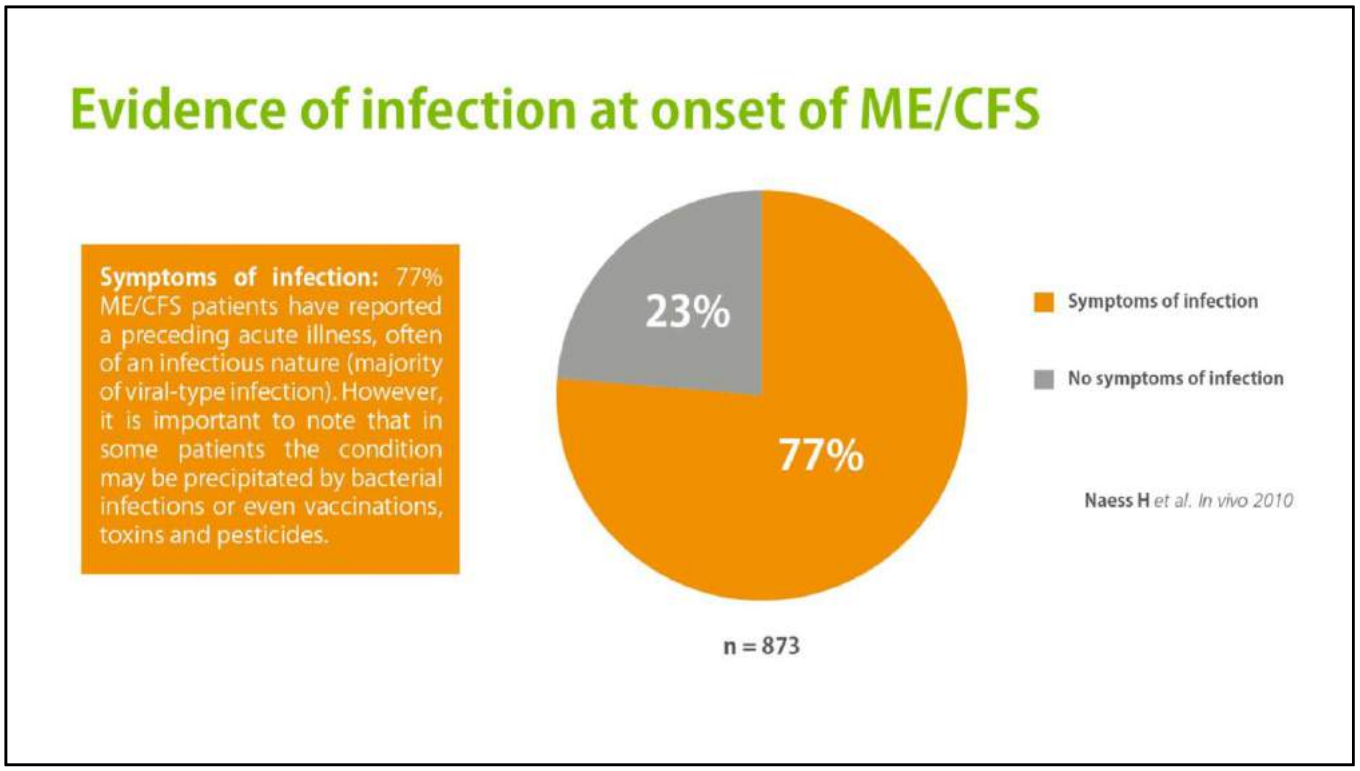


Various types of infections have been studied to determine if they might trigger ME/CFS. Researchers from around the world have studied if a single type of infection might be the cause of ME/CFS, and not yet found any association between ME/CFS and infection. Researchers are still analyzing samples from ME/CFS patients using the latest molecular methods to search for previously unknown infections (pathogen discovery). To date, these studies suggest that noone infection or pathogen causes ME/CFS and that the illness may be triggered by a variety of conditions.



Several pathogens have been proposed as infectious triggers:

1. Epstein-Barr virus infection, also known as mononucleosis
2. Human herpesvirus 6 infection, a virus that can cause problems for people withimpaired immune systems.
3. Enterovirus infection, a type of virus that enters through the gastrointestinal track and can have no symptoms, mild flu-like symptoms, or rarely severe and even deadly symptoms.
4. Rubella, a viral infection also known as German measles
5. *Candida albicans*, a fungus that causes yeast infections
6. Bornaviruses, which cause borna disease, an infectious neurological syndrome7- Mycoplasma, a cause of atypical pneumonia
7. Ross River virus, which causes Ross River Fever, a mosquito-borne tropical disease
8. *Coxiella burnetti*, the agent that causes Q fever
9. SARS-CoV-2 causing COVID-19 (Long COVID and ME/CFS into post-viral fatigue sytndrome)

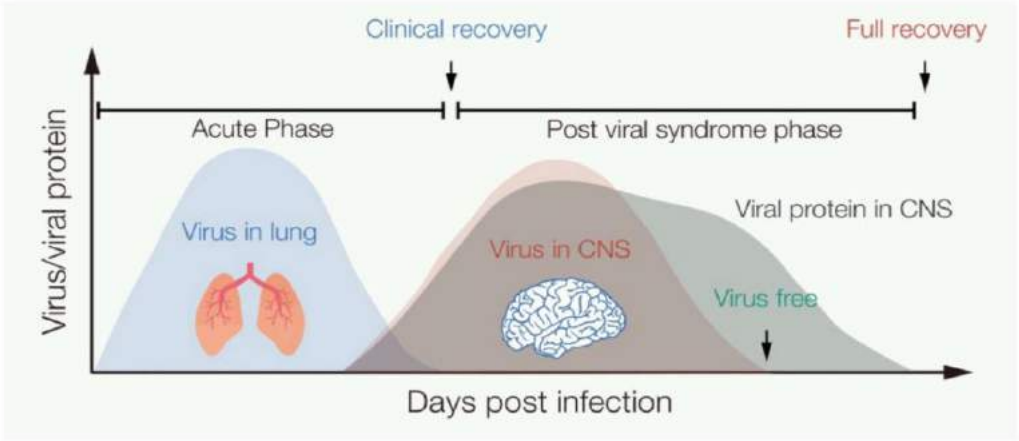


Some patients have reported a preceding acute illness, often of an infectious nature, such as a specific influenza like illness, streptococcal pharyngitis, acute EBV infection, gastroenteritis, glandular fever and sinusitis.

However, it should be noted that in some studies preceding illness information was patient-reported without corroborating laboratory investigations and most studies lacked a comparative control group. The possibility that the CFS/ME was triggered by an unspecified viral infection not be picked up by laboratory investigations can be acknowledged in discussions with patients and families but it should be emphasized that viral infection does not have to be the trigger or the cause.

It appears that the majority of people suffering from ME/CFS, generally can trace the onset of their illness to a viral-type illness. A recent series of studies has confirmed this, with 72% in the UK, 78% in a Japanese cohort, 86% in Holland, increasing to 91% in an American report. However, it is important to note that although viruses are often thought to be implicated, for some sufferers, the condition may be precipitated by bacterial infections or even vaccinations, toxinsand pesticides.

Post-viral fatigue syndrome following acute COVID-19 infection



This slide shows 2 phases of the natural clinical history of COVID-19.

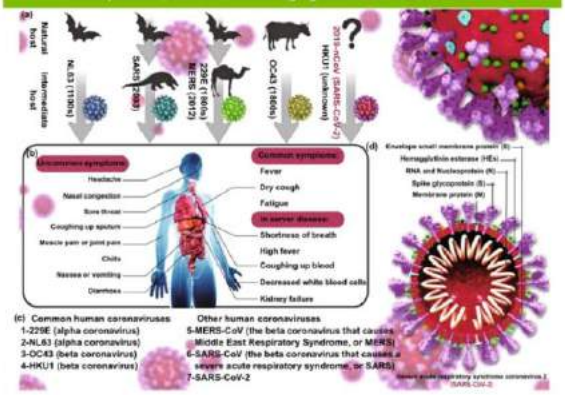
A first initial phase of acute SARS-CoV-2 infection in which a large majority of patients recover in a few weeks (80%). However, as shown in a second phase, a significant proportion of COVID-19 patients (20%) have not yet recovered after more than 12 months from COVID-19 infection and are experiencing long-term post-acute symptoms/sequelae (PASC) very similar to other post-viral fatigue syndromes (such as ME/CFS, influenza, glandular fever, Ebola, SARS-CoV and MERS).

Further research is needed to establish SARS-CoV-2 as an infectious trigger for Post-COVID fatigue syndrome and define prevalence, risk factors, underlying pathomechanisms and possible interventional strategies to treat this condition.

Post-Viral Fatigue Syndrome and *Long COVID*: lessons learned from past epidemics

1918 *"Spanish flu"* (H1N1-influenza virus): the largest pandemic of the 20th century

Other previous CoVs and new emerging SARS-CoV-2 variants in PVFS



A commonly reported symptom of COVID-19 is fatigue, and well documented evidence suggests that some people continue to experience severe and prolonged fatigue among other core symptoms as they recoverfrom this infection. This is not surprising, as post-infectious fatigue has been widely observed across a variety of other viral and non-viral infections.

In this slide we should learn about the long term effects of post- acute symptoms/sequelae from other past outbreaks and their implications for those with Long COVID.

The most devastating epidemic in modern history was the Spanish Flu of 1918, caused by the H1N1 influenza virus. Researchers estimate the global mortality of this pandemic was between 25 to 100 million deaths worldwide. Of those who survived, some experienced complications during recovery and others remained severely ill. Fatigue was one of the most common longer-term consequences of the Spanish flu.

Post-infectious fatigue has also been observed after the onsetof other epidemics such as 2003 SARS-CoV virus outbreak and 2012 MERS causing SARS epidemics.

Long COVID represents a subtype (clinical phenotype) within PVFS based on the clinical presentation and symptom evolution among patients. Long COVID is defined as a symptom cluster following acute SARS.CoV-2 infection in which fatigue is also the most common complaint and independent of severity of initial infection that shares more similarities than differences with other PVFS such as ME/CFS.

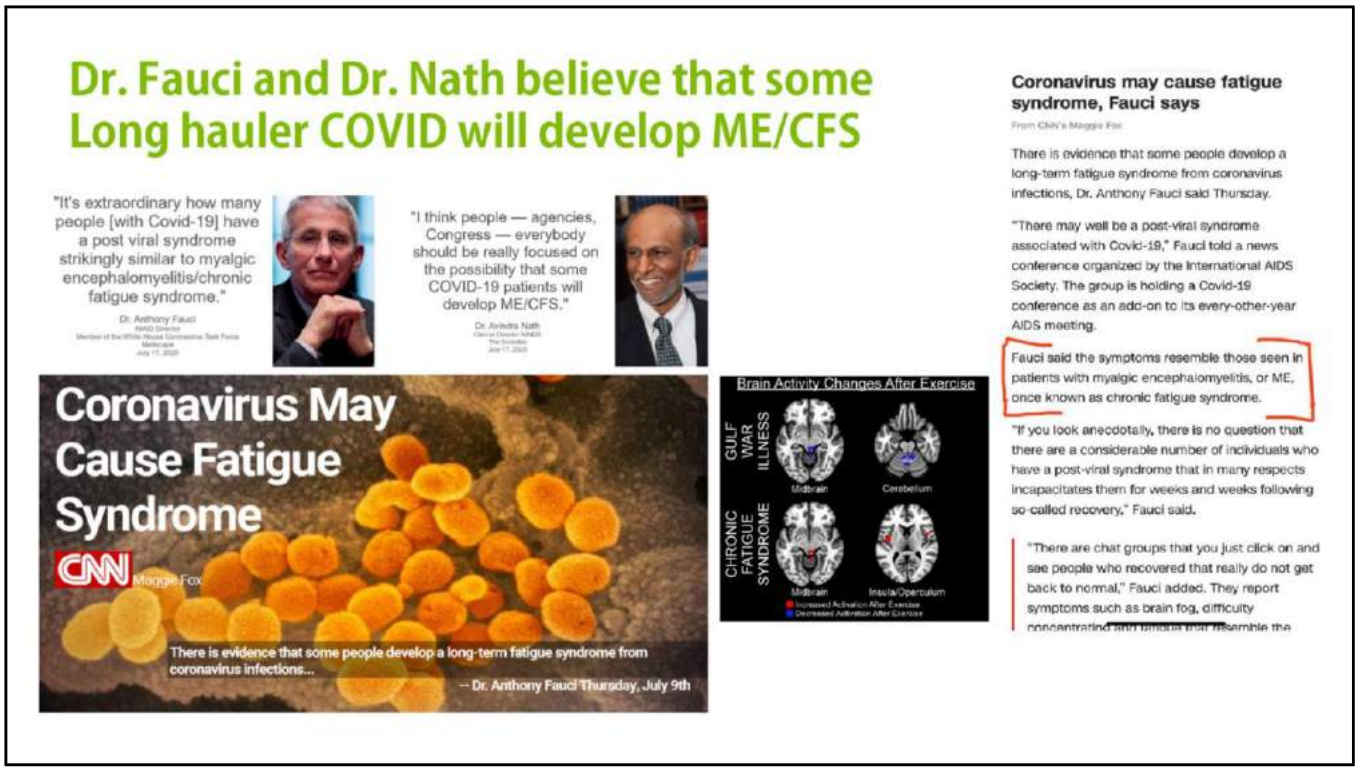
Common Disease Symptoms

Solve M.E.

SYMPTOM	Cold	Flu	Acute COVID-19	Long-Term COVID-19	ME/CFS
Fever, Feeling Feverish/Chills	Rare	Common	Common	Intermittent	Intermittent
Cough	Sometimes	Common	Common	Sometimes	Intermittent
Shortness of Breath/Difficulty Breathing	No	Rare	Common	Sometimes/ Intermittent	Intermittent
Fatigue (Tiredness)	Sometimes	Common	Common	Common	Persistent
Disabling Fatigue, Worsened by Activity*	No	No	Sometimes	Common	Persistent
Sore Throat	Common	Sometimes	Sometimes	?	Intermittent
Runny or Stuffy Nose	Common	Sometimes	Rare	No	No
Muscle/Joint Pain or Body Aches	Sometimes	Common	Common	Common	Persistent
Headache	Rare	Common	Sometimes	Sometimes	Intermittent
Sleep Disruption/Unrefreshing Sleep	No	No	No	Common	Persistent
Cognitive Disruption ("Brain Fog")	No	No	No	Persistent	Persistent
Loss or Taste or Smell	No	No	Common	Common	?
Large Swings in Heart Rate/Blood Pressure	No	No	No	Common	Common
Diarrhea or Vomiting	No	Sometimes	Sometimes	?	Sometimes

Long COVID defines a series of chronic symptoms that patients may experience after resolution of acute COVID-19 infection.

Early reports and recent systematic review (Wong et al. Medicina 2021) from studies with Long COVID patients suggests a constellation of common symptoms with high degree of similarities to ME/CFS. It is particularly notable that 12 of the 21 selected studies, suggested fatigue as a predominant symptom of Long COVID patients. However, there is currently insufficient evidence to establish COVID-19 as an infectious trigger for ME/CFS.



People with PVFS following well-documented acute infections share a group of common symptoms with ME/CFS. Many, but not all, people with ME/CFS note that it began suddenly, with an apparently infectious illness characterized by respiratory symptoms, fever, adenopathy, myalgia, and other symptoms. Yet the spectrum of symptoms in ME/CFS that follows an apparently infectious illness due to an undocumented infectious agent is very similar to the illness following a well-documented infectious agent.

Indeed, according to Dr. Fauci and Dr. Nath, patients with Long COVID (aka Long hauler COVID) can develop "a post-viral syndrome" that's very strikingly similar to ME/CFS.

As of May 2020, COVID-19 has been documented in about 165 million people, globally. Using similar estimates to those were used for the US, that number would be predicted to increase to nearly 110 million during 2021, and to generate over 10 million new cases of ME/CFS, globally.

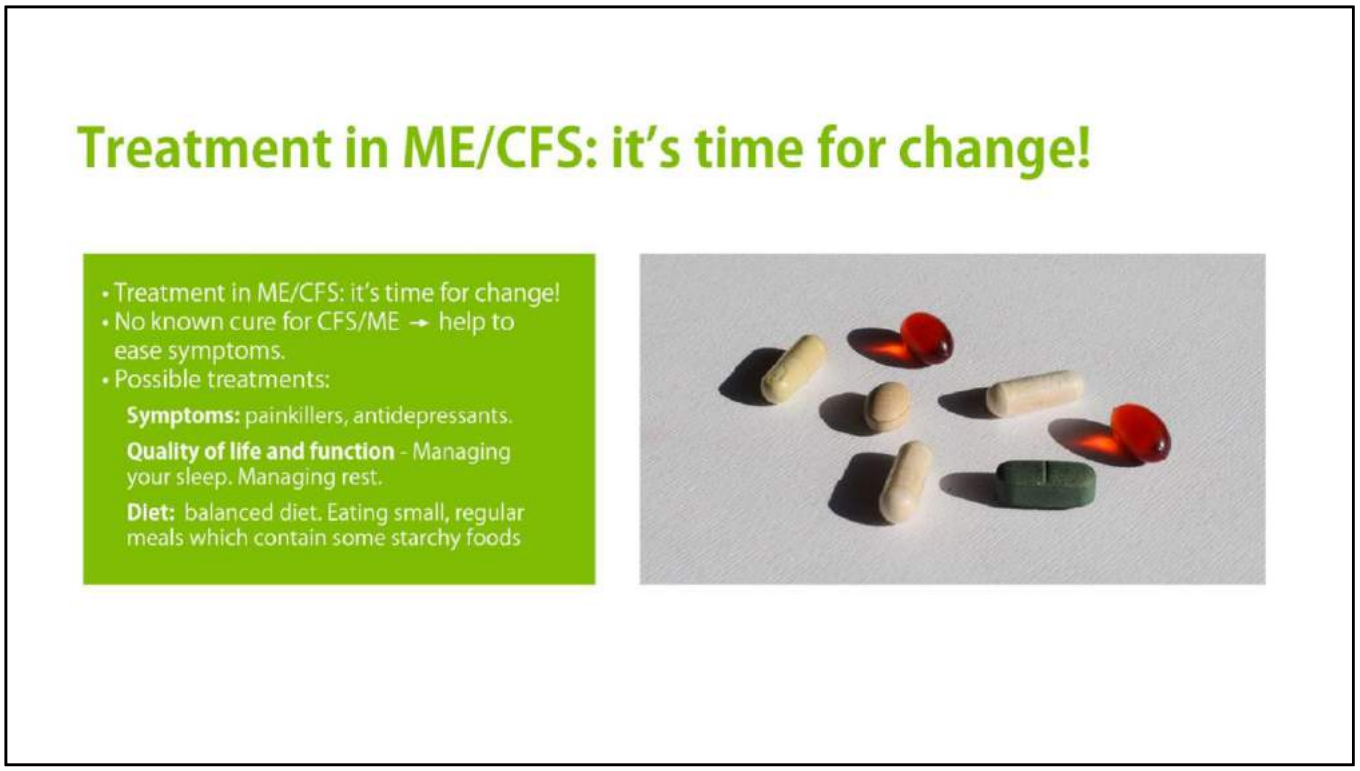
The Underlying Biology of ME/CFS

- Brain (Functional MRI/PET neuroimaging scans)
- Exercise (& post-exertional malaise)
- Immune system (& Infectious agents)
- Energy metabolism/mitochondria
- Epigenic studies (EGWAS in B, T & NK immune cells)
- A Hypothesis: Low-grade brain inflamation, often triggered by brain-immune-gut connections)

In the last few years, although funding has been a big problem in Spain for this illness, we have advanced in ME/CFS research based off of these 5 emerging or hot topics of the field.

Our working hypothesis is that a low grade neuroinflammation could possibly be triggered by dysfunction in glia cells, immune cells, and the gut interaction with each other.

- ME/CFS is a complex, multisystemic, debilitating illness characterized by persistent fatigue lasting 6 months and post-exertional malaise as hallmark symptom, among others
- Chronic fatigue does not improve with rest. It worsens with physical activity and mental effort during weeks, and even months
- Inability to participate in daily activities
- It affects more women (90%) of childbearing age
- Not yet identified the cause. No specific "blood test" nor FDA-approved drugs for treatment exist



Treatment in ME/CFS: it's time for change. There is no known cure for CFS/ME although current treatments may help to ease symptoms.

Treatments that may be considered include the following:

- **Management of your symptoms:** painkillers, antidepressants.
- **Management of your quality of life:**
    - **Diet:** It is very important that patients have a well- balanced diet. Eating small, regular meals which contain some starchy foods is often beneficial.

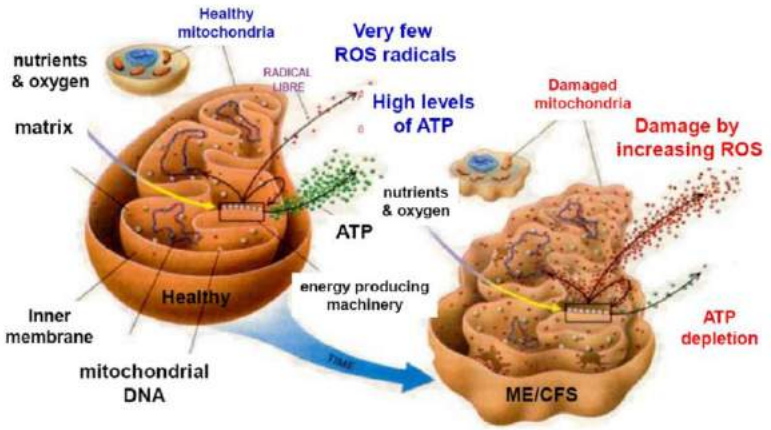
    - **Restful sleep**

ReConnect®

Mitochondria as therapeutic target in ME/CFS (**ReConnect** *study*)

In Spain we are working in nutritional supplements associated to the functional capacity of the mitochondria in ME/CFS. We have carried out a pilot RCT (Reconnect study) which aims to looking for the **effect of the oral supplementation of CoQ10 plus NADH on the relief of fatigue in ME/CFS.**

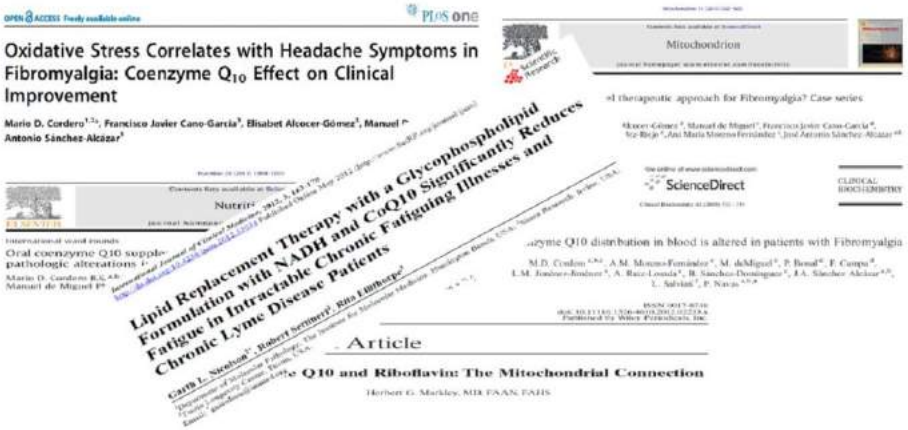
Mitochondrial dysfunction and oxidative stress in ME/CFS: a *"cellular bioenergetic crisis"*



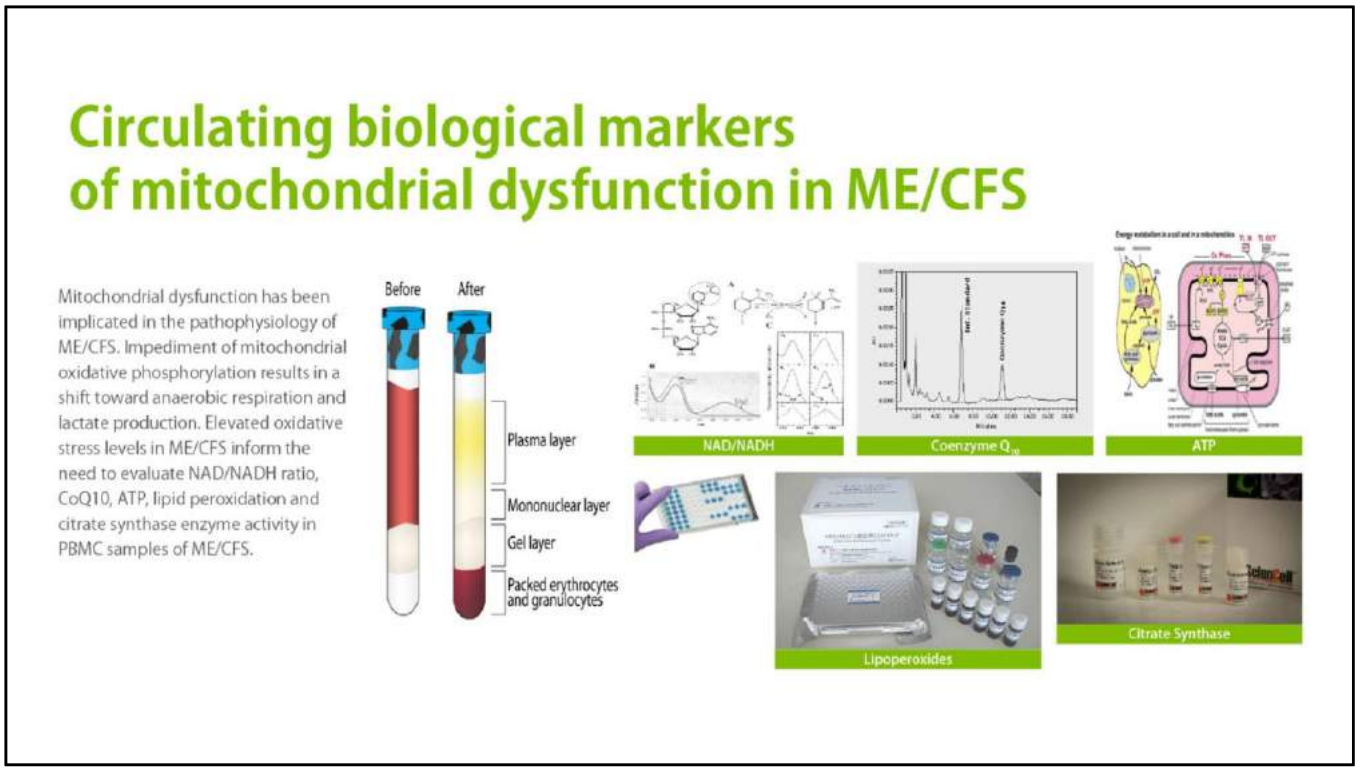
Numerous research teams on ME/CFS have reported mitochondrial dysfunction (such as depleted ATP production, defective oxidative phosphorylation, elevated lactate, lowered CoQ10 levels, and dysregulated mitochondrial dynamics/function) both in PBMC and muscle biopsies from patients with ME/CFS, which is indicative of significant impaired oxidative stress.0 plus NADH on the relief of fatigue in ME/CFS.

Clinical trials with NADH supplementation in ME/CFS

Clinical trials with Coenzyme Q_{10} supplementation in ME/CFS



CoQ10 may work in ME/CFS because to its antioxidant properties and its ability to enhance cellular energy production. ME/CFS studies consistently show increased rates of oxidative stress. CFS/ME pilot studies: Several researchers found highly reduced levels of CoQ10 associated with increased symptoms in ME/CFS. CoQ10 has been used in combination with other antioxidants. Early study suggests that it may have antioxidant benefits in people with chronic conditions. So, more information is needed on the potential benefits of CoQ10 alone and in combination with other antioxidants (NADH). Early research shows that CoQ10 may benefit and improve symptoms of CFS/ME and Fibromyalgia.



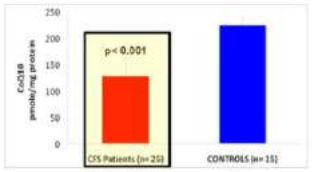
Mitochondrial dysfunction has been implicated in the pathophysiology of ME/CFS. Impediment of mitochondrial oxidative phosphorylation results in a shifttoward anaerobic respiration and lactate production.

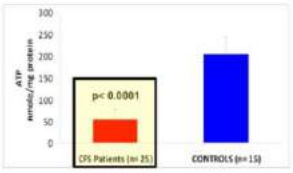
Elevated oxidative stress levels in ME/CFS inform the need to evaluate NAD/NADH ratio, CoQ10, ATP, lipid peroxidation and citrate synthase enzyme activity in PBMC samples of ME/CFS.

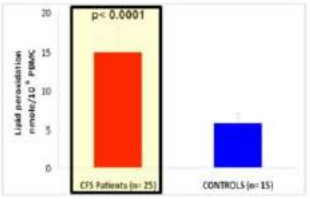
Baseline levels of Coenzyme Q_{10}, ATP and lipoperoxides in Spanish ME/CFS patients

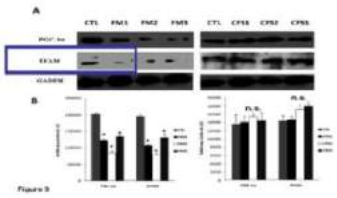
Disorders marked by oxidative stress cause large shifts in the amounts of ubiquinone & ubiquinol in the body possible association between the CoQ10 (ubiquinone), ATP and lipoperoxides levels in CFS patients.

- Low levels of CoQ10 and ATP on CFS patients compared to controls.
- High levels of lipoperoxides on CFS patients compared to controls (indicative of oxidative stress).
- Hypothesis: mitochondrial dysfunction could be a biomarker between different CFS subtypes, indicating the mitochondria as a new potential therapeutic target for this condition.









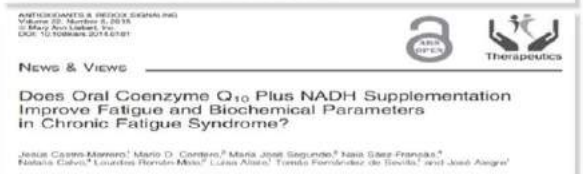
Castro J *et al. ARS 2013*

Disorders marked by oxidative stress cause large shifts in the amounts of ubiquinone & ubiquinol in the body. We have investigated the possible association between the CoQ10 (ubiquinone), ATP and lipoperoxides levels in PBMC from 25 Spanish CFS patients and 15 non-fatigue controls. PBMC showeddecreased levels of CoQ10 and ATP from CFS patients compared to controls. On the contrary, CFS patients had significantly increased levels of lipoperoxides compared to controls that were indicative of oxidative stress.

These data lead to the hypothesis that mitochondrial dysfunction events could be a biomarker between different CFS subtypes, indicating the mitochondria as a new potential therapeutic target for this condition.


News & Views

Could Mitochondrial Dysfunction Be a Differentiating Marker Between Chronic Fatigue Syndrome and Fibromyalgia?


News & Views

Does Oral Coenzyme Q_{10} Plus NADH Supplementation Improve Fatigue and Biochemical Parameters in Chronic Fatigue Syndrome?


Clinical Nutrition

Randomized control trials

Effect of coenzyme Q_{10} plus nicotinamide adenine dinucleotide supplementation on maximum heart rate after exercise testing in chronic fatigue syndrome — A randomized, controlled, double-blind trial

Study population

Coenzyme Q_{10} + NADH
(n = 39)

Placebo
(n = 34)

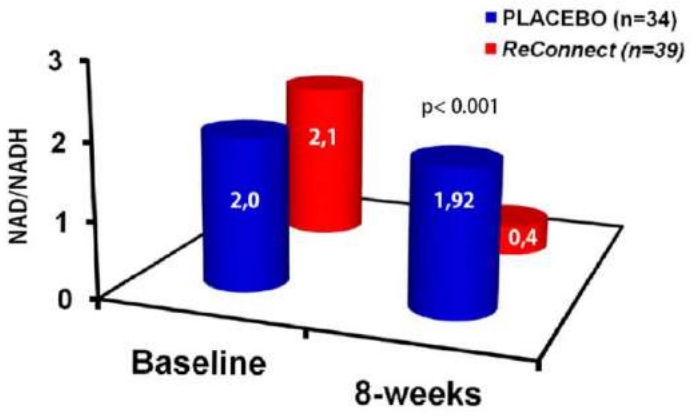
73 ME/CFS patients (80% had Fibromyalgia)
All were female (mean age: 49.3 yrs)

ME/CFS Clinical Unit (Vall d'Hebron Hospital, Barcelona, Spain)
From Jan to Dec 2013
Randomized controlled trial: CoQ10 (200 mg/day)
NADH (20 mg/day)

A total of 73 consecutive eligible female Spanish ME/CFS patients (mean age: 49 years) who attended to ME/CFS Clinical Unit at Vall d'Hebron Hospital (Barcelona, Spain) from January to December 2013 with a diagnosis of ME/CFS according to 1994 CDC/Fukuda criteria were initially recruited and enrolled in the study.

NAD+/NADH levels in ME/CFS

- NAD+/NADH ratio regulates the intracellular redox status
- Significant decrease in the NAD+/NADH ratio in ReConnect patients after 8 weeks
- **Conclusion:** ReConnect stimulates the production of NADH providing increased energy level and improved mitochondrial function in CFS patients.



(Castro-Marrero J et al. ARS 2015)

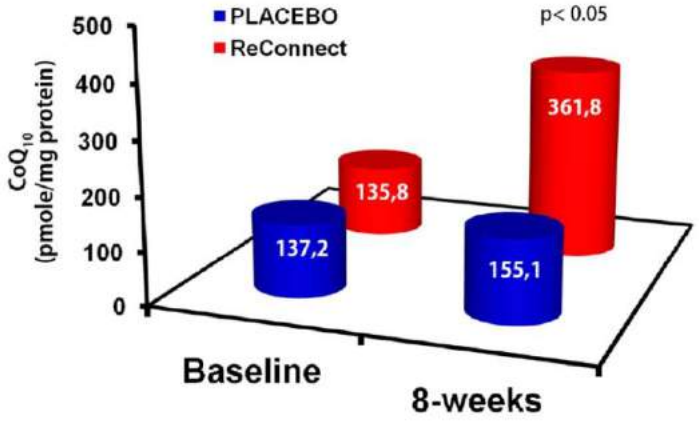
The NAD/NADH ratio plays an omnipresent role in regulating the intracellular redox status, and therefore represents a function of the metabolic state.

To our knowledge, there is no information well documented regarding the role of NAD and NADH in CFS.

In this slides we can see a significant decrease in the NAD/NADH ratio in ReConnect patients after 8 weeks. All this demonstrates that the ReConnect stimulates the production of NADH providing increased energy level and improved mitochondrial function in CFS patients.

Coenzyme Q10 levels in ME/CFS

- CoQ10 is essential for the production of cellular energy
- CFS patients treated showed a significant increase in CoQ10 levels ($p< 0.05$) and a significant decrease in lipoperoxides levels ($p< 0.05$) comparing with placebo group
- **Conclusion:** ReConnect slows induction of mitochondrial ROS radicals and damage by oxidative stress in treated patients improving symptoms.



(Castro-Marrero J et al. ARS 2015)

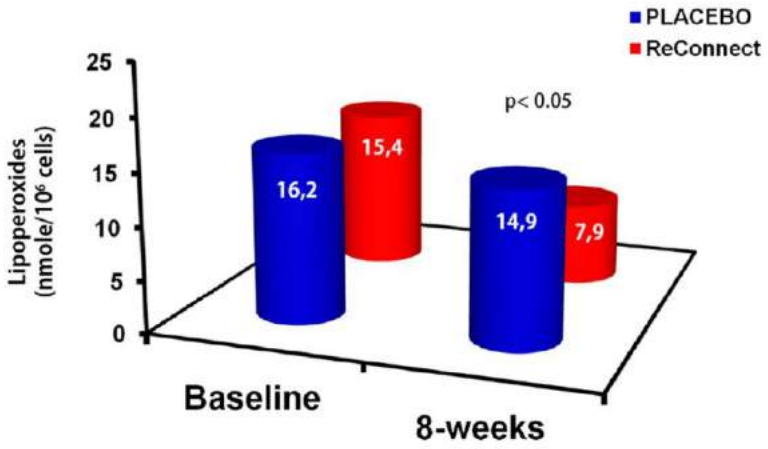
Because CoQ10 is essential for the production of cellular energy, supplementing it enhances stamina and health.

Millions of people have taken CoQ10 supplements with no reported toxicity in over a thousand human and clinical trials.

In this study, we measure CoQ10 and lipoperoxides levels in PBMC from CFS patients treated with ReConnect versus placebo. We note that CFS patients treated showed a significant increase in CoQ10 levels ($p< 0.05$) and a significant decrease in lipoperoxides levels ($p< 0.05$) comparing with placebo group**. All these results means that the *ReConnect* slows induction of mitochondrial ROS radicals and damage by oxidative stress in treated patients improving symptoms.**

Lipoperoxides in ME/CFS

- CFS patients treated with ReConnect for 8-weeks showed a significant decrease in lipoperoxides levels ($p < 0.05$) comparing with placebo group
- **Conclusion: ReConnect** slows production of mitochondrial ROS radicals and damage by oxidative stress in treated patients improving symptoms



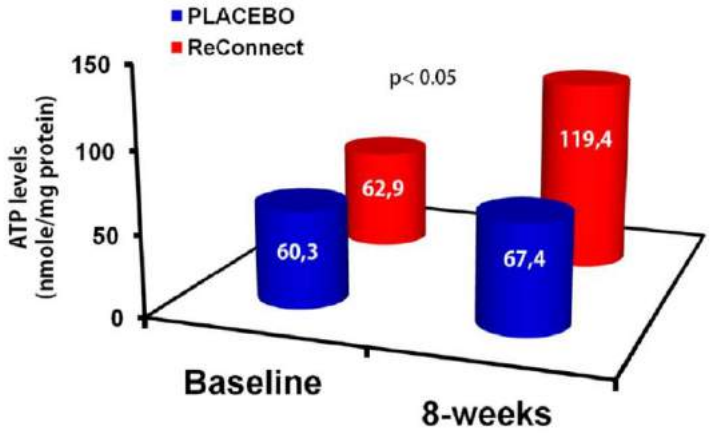
(Castro-Marrero J et al. ARS 2015)

In this study, we measure lipoperoxides levels in PBMC from CFS patients treated with ReConnect vs. placebo.

We note that CFS patients treated with ReConnect for 8-weeks showed a significant decrease in lipoperoxides levels ($p<0.05$) comparing with placebo group. **All these results show that the *ReConnect* slows production of mitochondrial ROS radicals and damage by oxidative stress in treated patients improving symptoms.**

ATP levels in ME/CFS

- **ATP levels:** indicator of cellular bioenergetic status
- CFS patients treated with ReConnect showed higher levels of mitochondrial ATP production compared to placebo (p< 0.05) suggesting that mitochondrial power of energy production may be reduced in CFS patients. In order to verify this, mitochondrial citrate synthase activity in both intervention groups was measured.



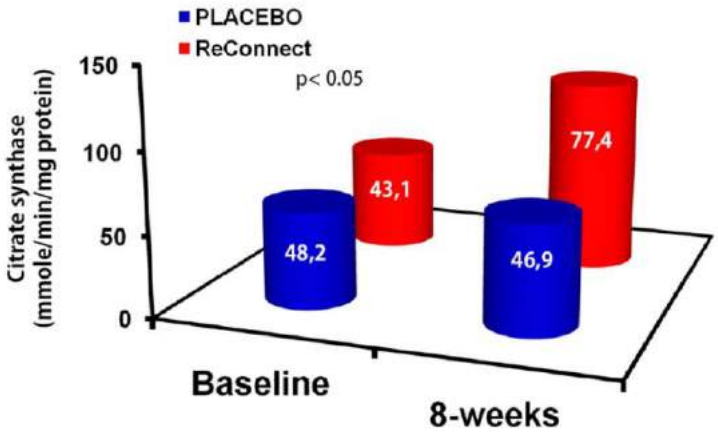
(Castro-Marrero J et al. ARS 2015)

As an indicator of cellular bioenergetic status, we measured total ATP levels in PBMC from CFS patients. CFS patients treated with ReConnect showed higher levels of mitochondrial ATP production compared to placebo (p< 0.05) suggestingthat mitochondrial power of energy production may be reduced in CFS patients.

Citrate synthase activity in ME/CFS

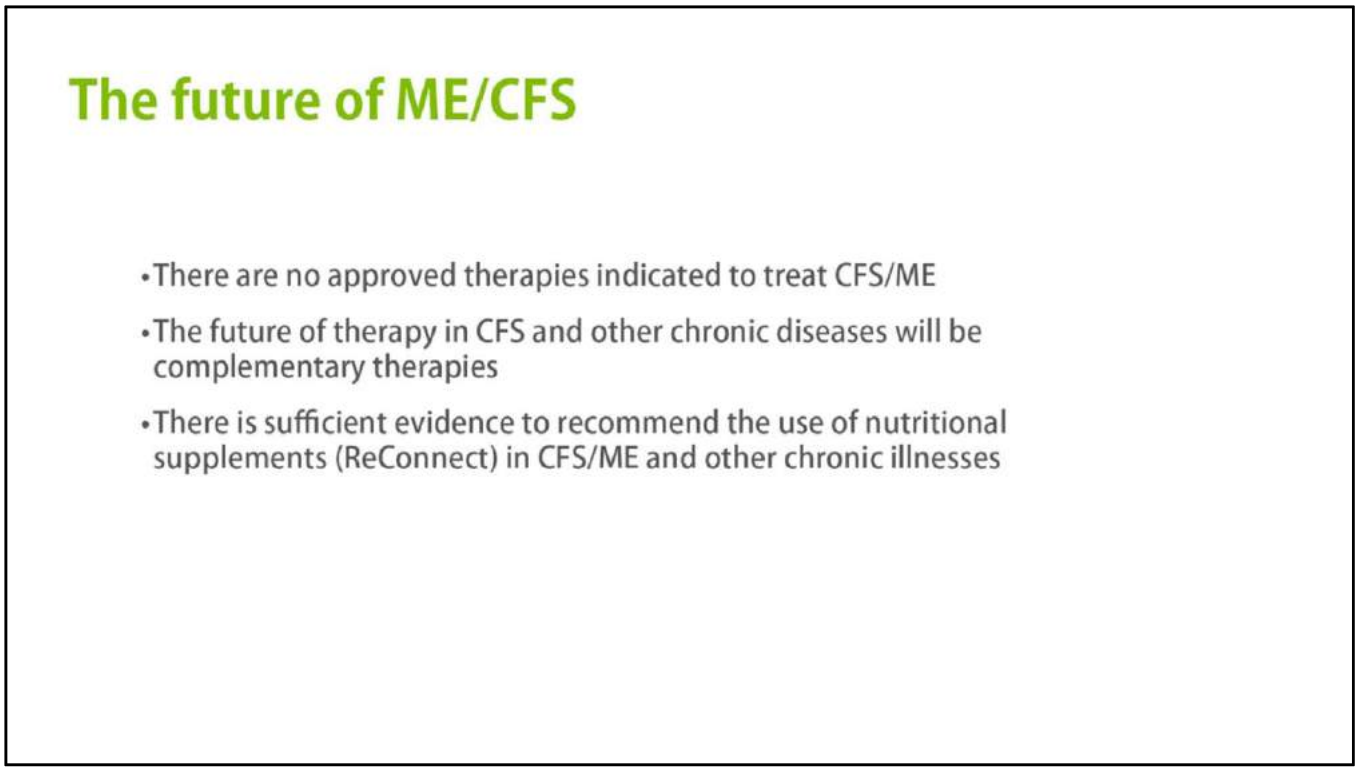
- Significant increase in PBMC citrate synthase in CFS patients treated with ReConnect vs. placebo (p< 0.05)

These data shown differences levels in mitochondrial mass content (more mitochondria) in CFS patients treated with ReConnect comparing placebo



(Castro-Marrero J et al. ARS 2015)

Surprisingly, **we observed a significant increase in PBMC citrate synthase in CFS patients treated with ReConnect vs. placebo (p< 0.05).** These data shown differences levels in mitochondrial mass content (more mitochondria) in CFS patients treated with ReConnect comparing placebo. In order to verify this, we measured mitochondrial citrate synthase activity in both intervention groups.



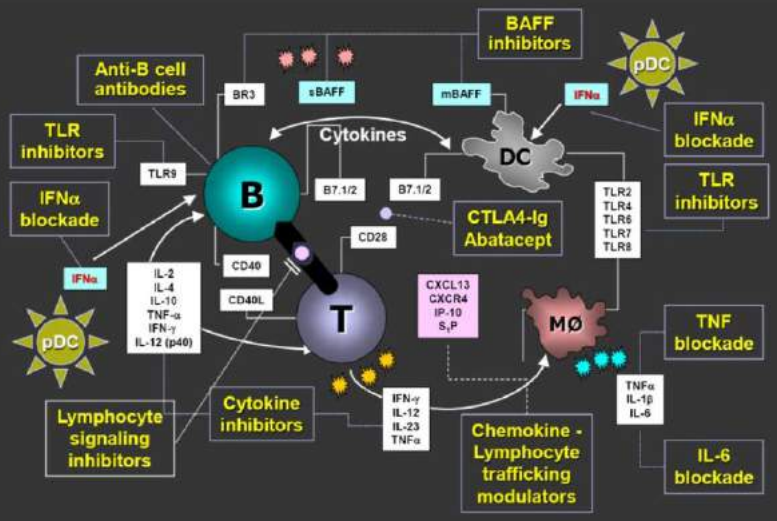
CFS/ME is a serious disease and there are no approved therapies indicated to treat CFS/ME. The lack of approved therapies indicated for the treatment of CFS/ME represents a public health concern.

What would be the future of therapy in CFS and other chronic diseases?

Complementary therapy: As there is only limited success with conventional treatments, it is understandable that people turn to complementary practitioners. Many people with CFS/ME find various therapies helpful. However, there is not enough research evidence to support the use of complementary therapies for the treatment of CFS/ME.

This Spanish RCT clinical study, implies sufficient evidence to recommend the use of nutritional supplements (ReConnect) in CFS/ME and other chronic illnesses.

Potential treatments for ME/CFS to 21st century



Barcelona Working Team on ME/CFS

Dr Jesus Castro
Dr. Jose Alegre
Vitae Health Innovation
info@vitaeinternational.com

UAB Universitat Autònoma de Barcelona

Vall d'Hebron Barcelona Hospital Campus

Vall d'Hebron Institut de Recerca

Thank you!

INNOVATIVE SME

VITAE