

Long Covid and Chronic Fatigue Shared Pathophysiology and Clinical Perspective



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Today's Discussion



Impact of dysfunctional adrenal physiology and novel clinical considerations.

- ***Mitochondrial dysfunction can impair cortisol synthesis***, contributing to symptoms like fatigue, brain fog, and reduced stress resilience.
- **Oxaloacetate** as a clinical intervention for Long Covid Symptoms.
- ***Low NAD+ levels are correlated with stress-related conditions***, impairing mitochondrial function, lowering ATP yield, and perpetuating fatigue.
- ***NAD+ (nicotinamide adenine dinucleotide) is a vital coenzyme*** that fuels mitochondrial energy production.

A recap of steroidogenesis starting with cholesterol and pregnenolone, and the role of the mitochondrion.

Long COVID Symptoms

- Long COVID is a chronic condition in which people suffer from an array of symptoms months or years after the acute phase of COVID.
- **According to 2024 statistics**, an estimated 7% of adults in the United States (approximately 17 million people) **were experiencing long COVID during that time.**
- More than 70% of COVID survivors suffer from ongoing symptoms four months post-infection.

Common Long Covid Symptoms

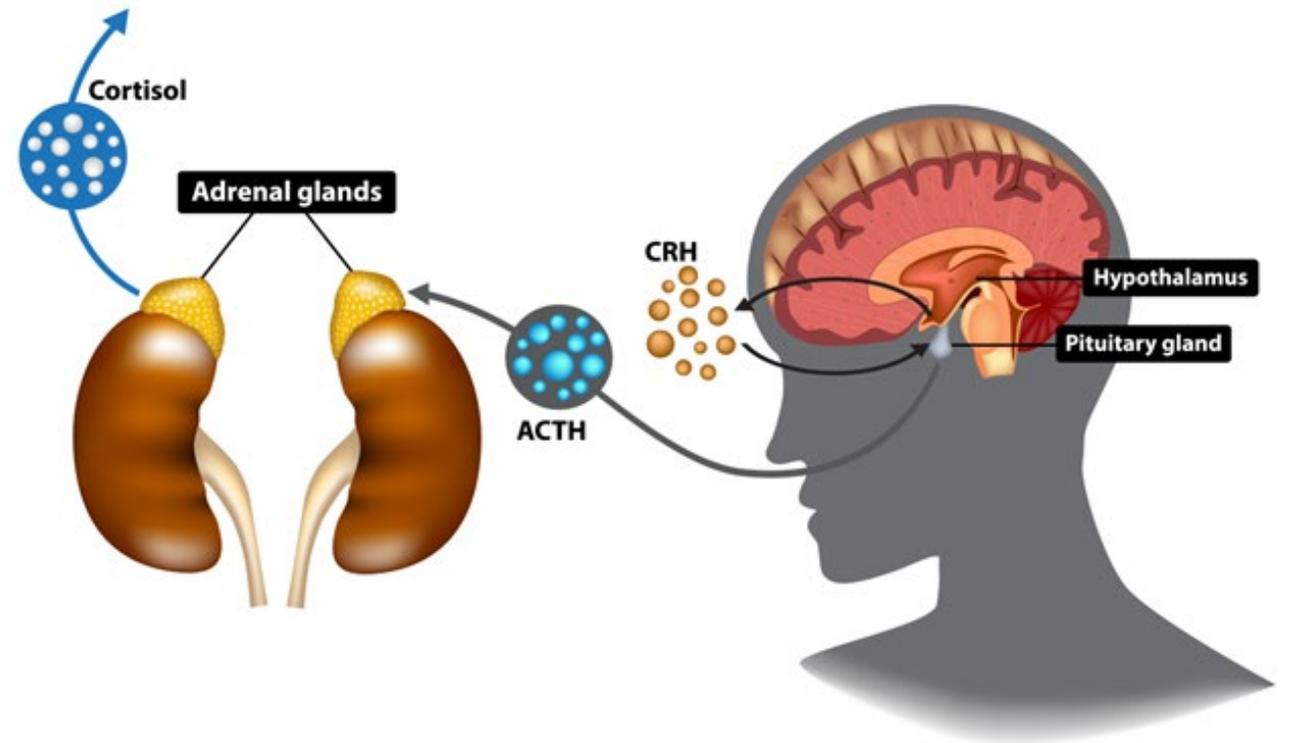
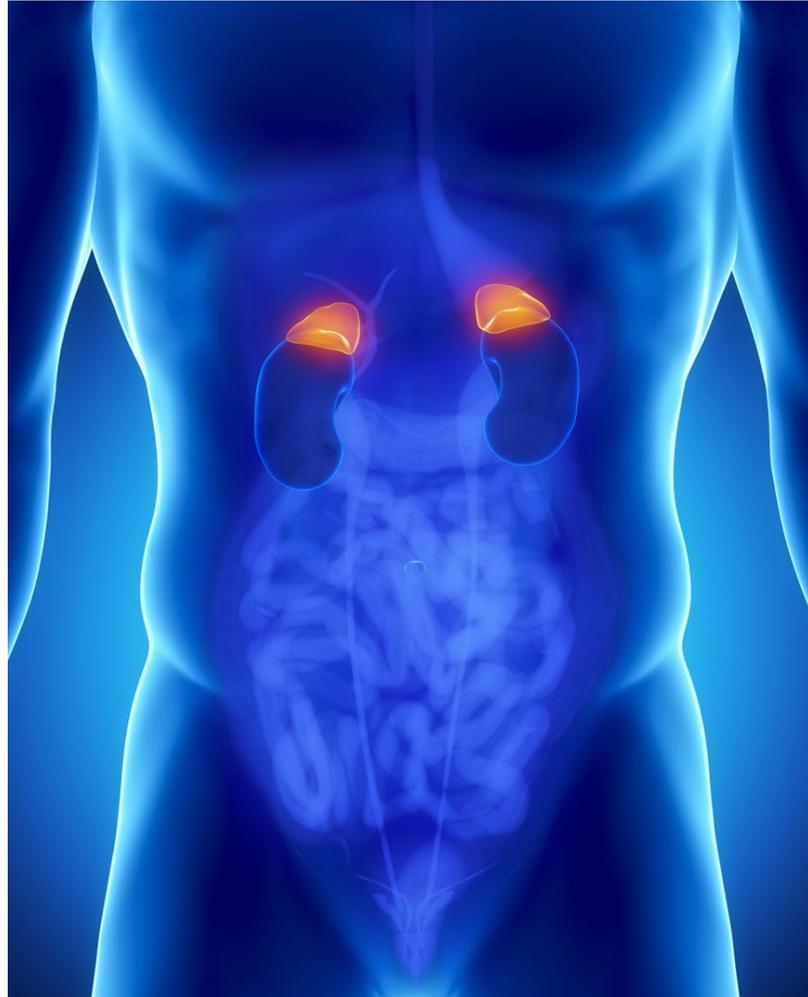
Table 1: Common Symptoms of Long COVID

General Symptoms	<ul style="list-style-type: none">• Fatigue and post-exertional malaise• Tiredness that interferes with daily life• Fever
Respiratory and Heart Symptoms	<ul style="list-style-type: none">• Difficulty breathing or shortness of breath• Coughing• Chest pain• Fast-beating or pounding heart (heart palpitations)
Neurological Symptoms	<ul style="list-style-type: none">• Difficulty thinking or concentrating (sometimes referred to as “brain fog”)• Headaches• Sleep problems• Dizziness upon standing (lightheadedness)• Pins-and-needles feelings• Change in ability to smell or taste• Depression or anxiety
Digestive Symptoms	<ul style="list-style-type: none">• Diarrhea• Stomach pain• Constipation
Other Symptoms	<ul style="list-style-type: none">• Joint or muscle pain• Rash• Changes in menstrual cycles

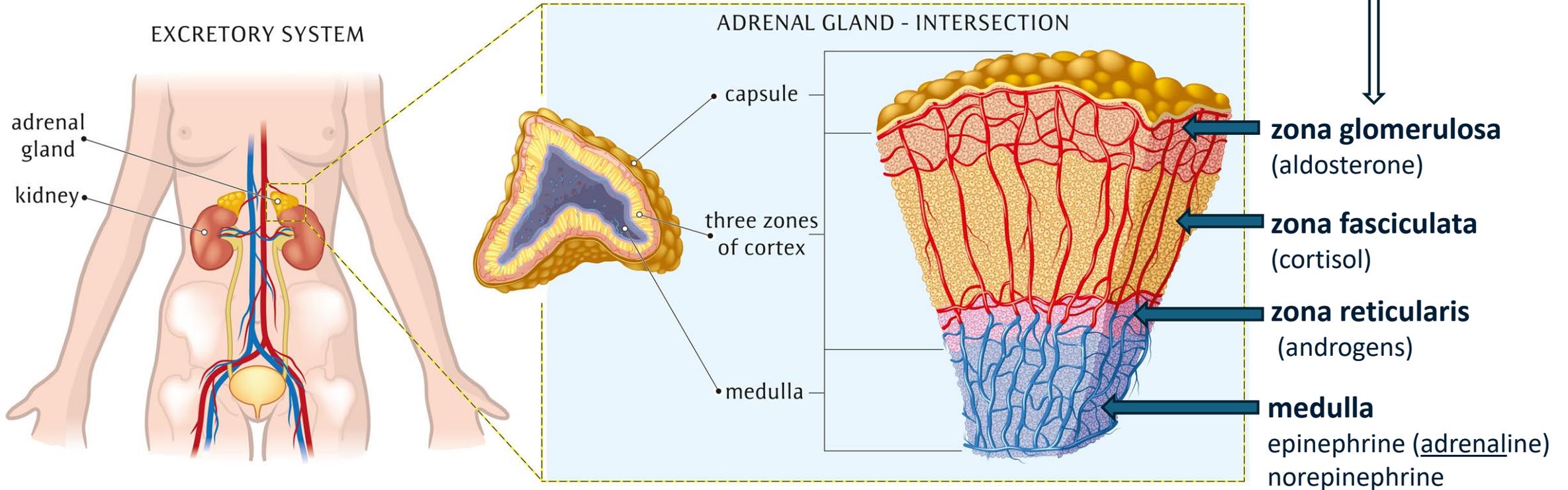
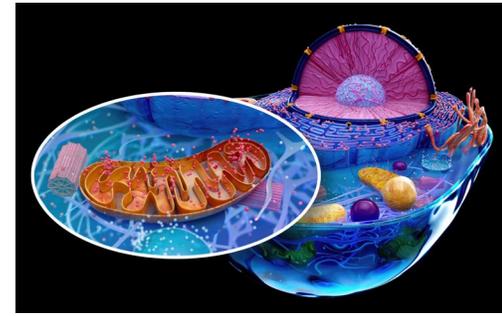
Mitochondrial Dysfunction a Common Denominator

- Long COVID, Adrenal Fatigue and Chronic Fatigue Syndrome (CFS) **share overlapping pathophysiological mechanisms, with mitochondrial dysfunction** emerging as a central driver of:
 - Persistent fatigue
 - Cognitive impairment (brain fog)
 - Multisystem dysregulation
- Mitochondrial dysfunction leads to cellular energy deficits, **oxidative stress, immune dysregulation, metabolic disturbances, and endothelial dysfunction, all states that could play a role in long COVID.**

Stress Triggers a Whole-Body Hormonal Cascade



Stress Serves as a Catch-22 Systemic Disruptor



Low Cortisol and Dysfunctional Adrenal Medulla Response

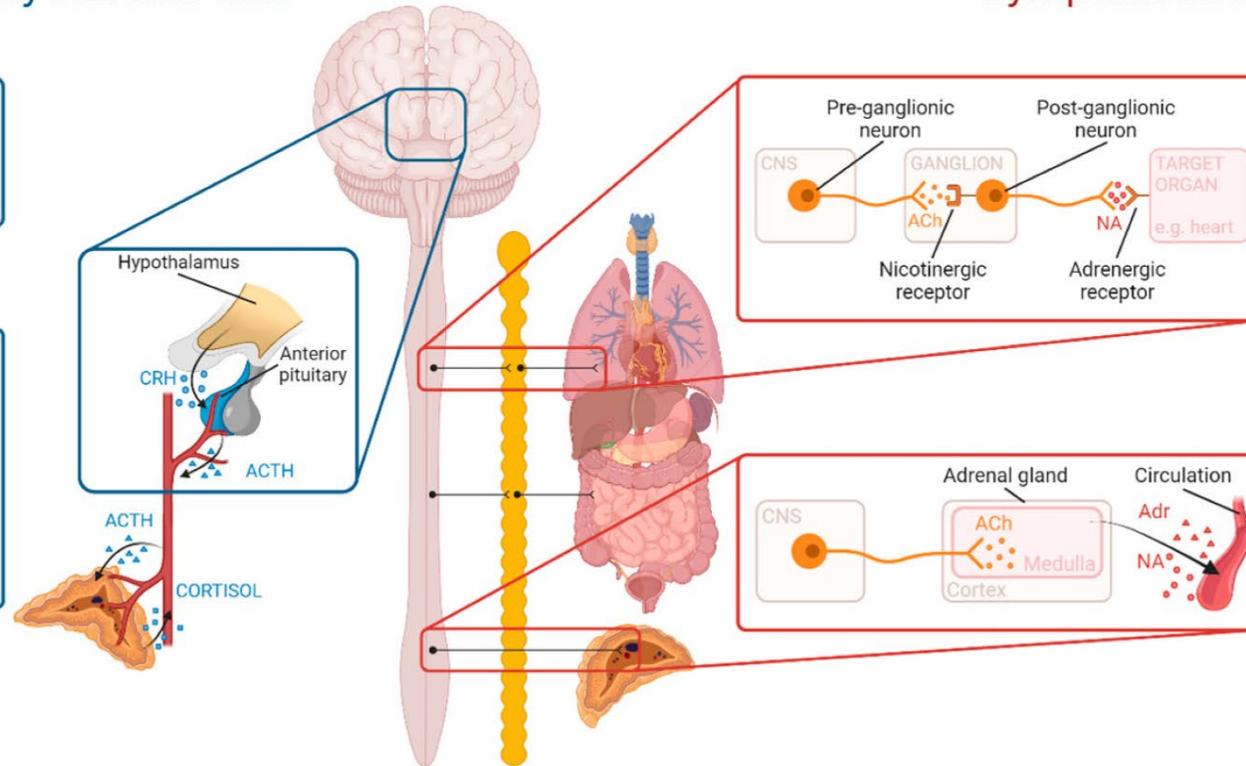
Hypothalamus-Pituitary-Adrenal axis

Dysregulations

- Hyper- & hypo-cortisolism
- Blunted cortisol response to stress
- GR expression

Pathophysiology

- Cortisol level ↓
 - ↑ pain sensitivity
 - ↓ pain threshold
 - ↓ cognitive function
- CRH antagonist
 - ↓ pain intensity
- Genetic polymorphisms
 - predictive for chronic pain



Sympathetic nervous system

Dysregulations

- Sympathetic dominance at rest
 - ↓ HRV
 - ↑ catecholamines
- Blunted stress response
- Most pronounced in CWP

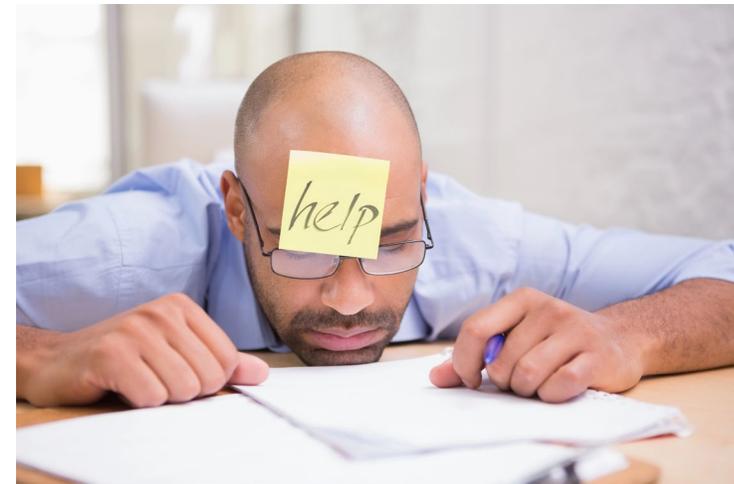
Pathophysiology

- Sympathetic dominance
 - ↓ cognitive function
 - ↑ pain intensity
- Genetic polymorphism
 - ↓ COMT & MAO-A&B enzymatic activity associated to ↑ pain intensity and stress
- Stress-related COMT mRNA
 - ↓ working memory

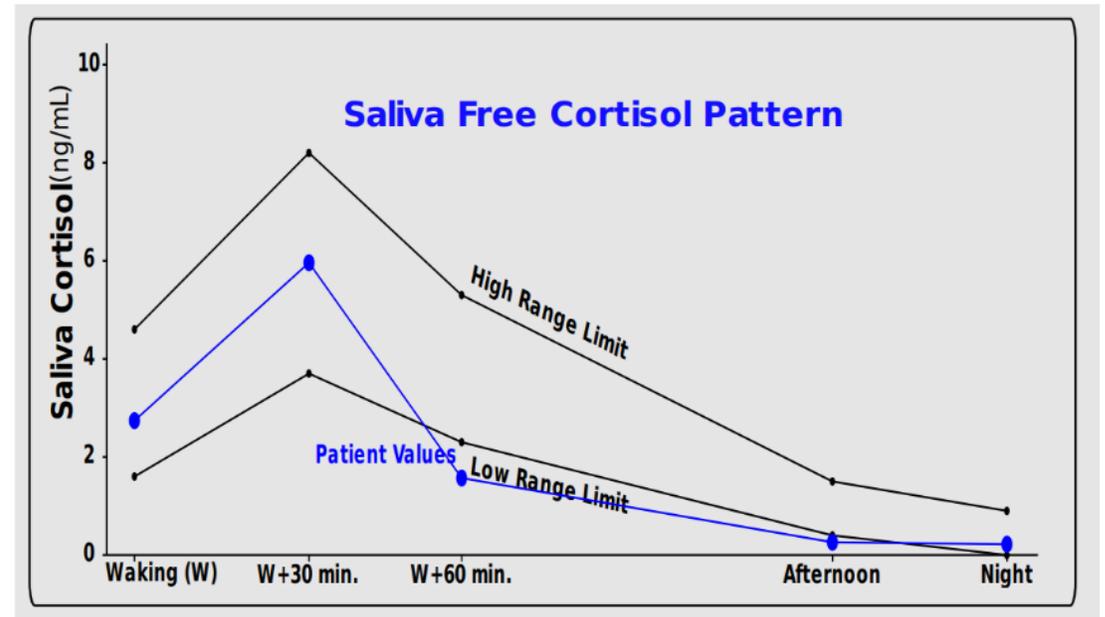
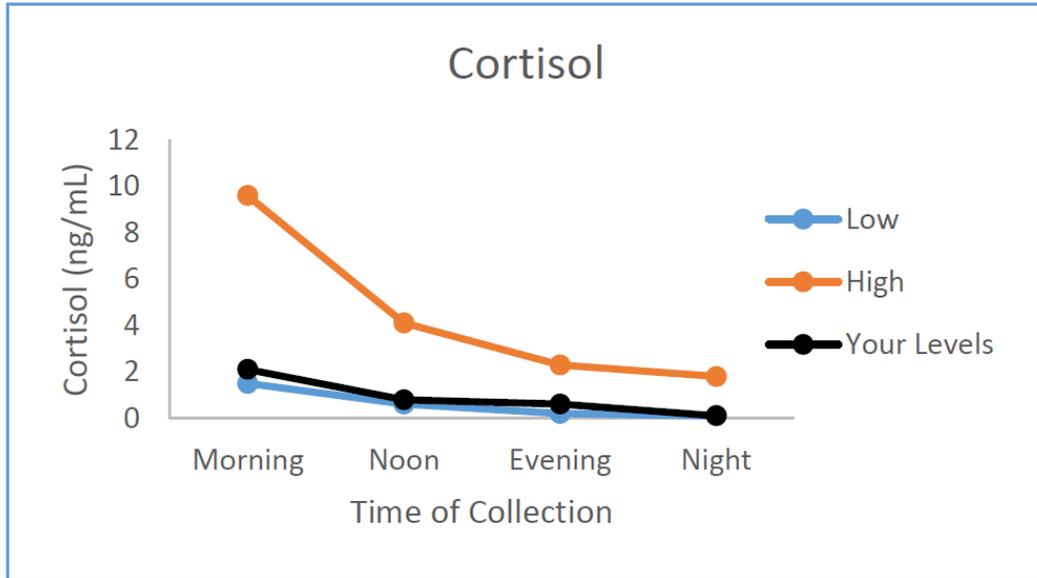
The Many Faces of Hypocortisolism

This phenomenon, termed ‘hypocortisolism’, has been reported in about 20–25% of patients with stress-related disorders.

- Chronic fatigue syndrome (CFS)
- Chronic pelvic pain (CPP)
- Fibromyalgia (FMS)
- Post-traumatic stress disorder (PTSD)
- Irritable bowel syndrome (IBS)
- Low back pain (LBP)
- Burnout
- Atypical depression

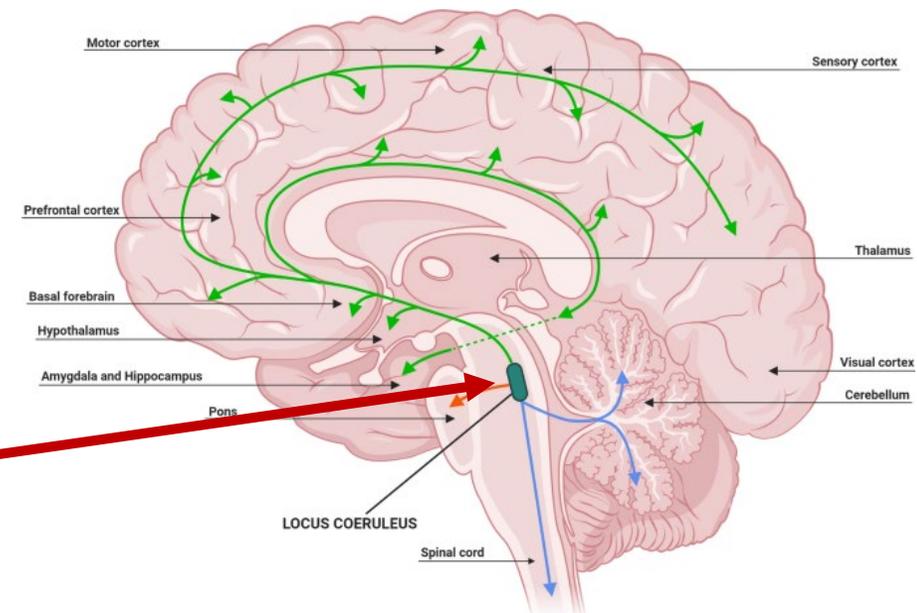
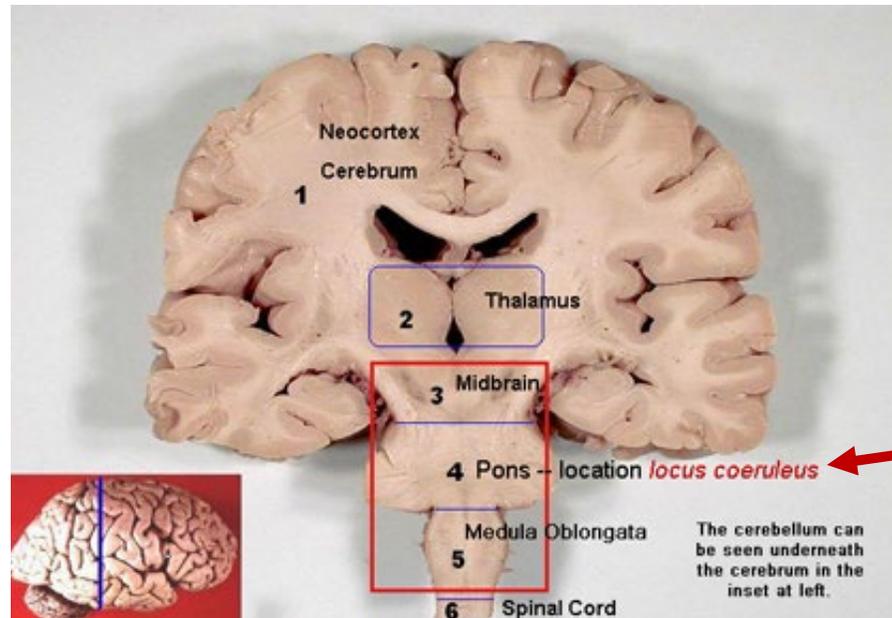


Patient Clinical Presentations of Cortisol



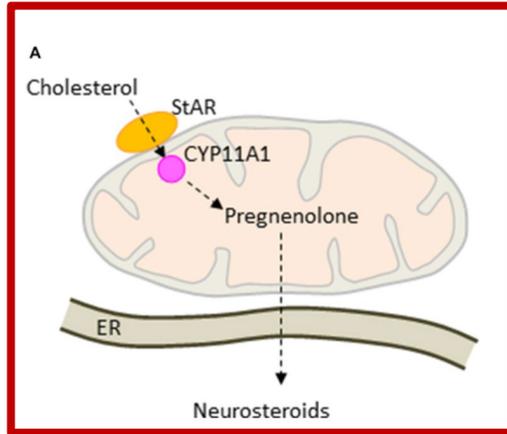
Wired and Tired

- The HPA axis plays an important role in the regulation of the SNS.
- **CRF (corticotropin-releasing factor) increases the spontaneous discharge rate of locus coeruleus (LC) neurons and enhances norepinephrine (NE) release in the prefrontal cortex** whereas glucocorticoids seem to exert more inhibitory effects on NE release. ←

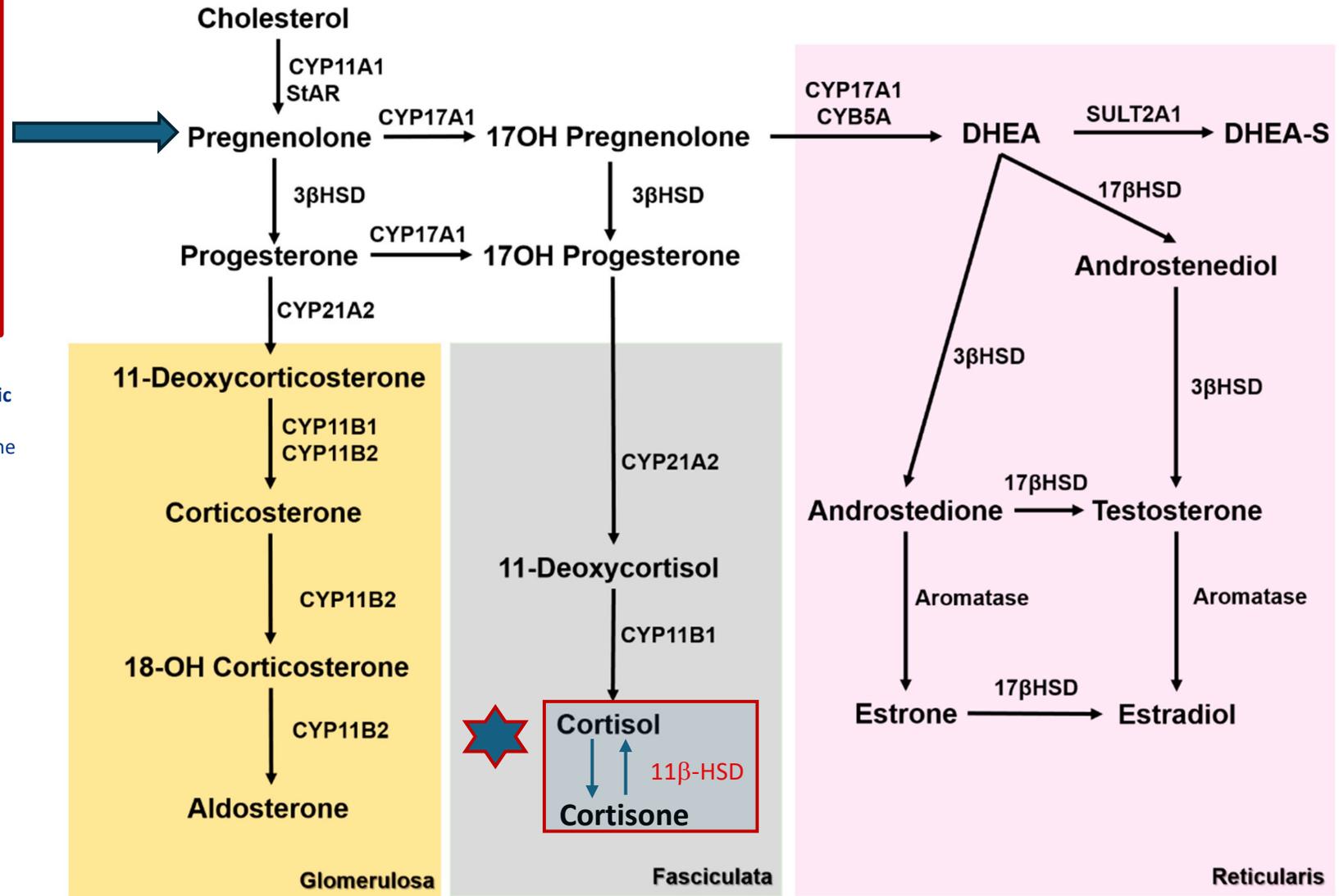


Locus coeruleus projections release norepinephrine throughout the central nervous system.

Functional Mitochondria are Essential for Steroidogenesis

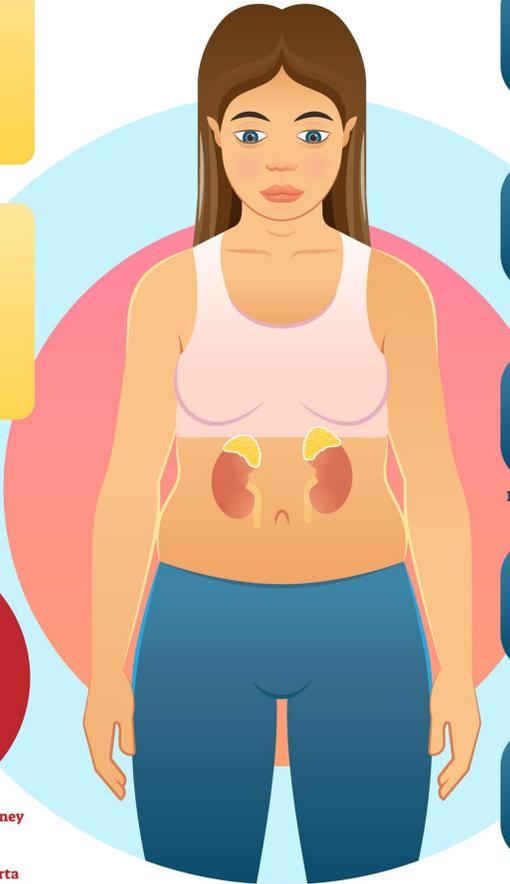
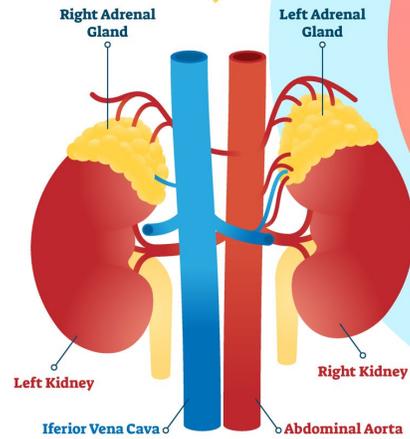


Cholesterol is translocated across the mitochondrial membrane by the steroidogenic acute regulatory protein (StAR). Thereafter, cholesterol is converted to pregnenolone by the side-chain cleavage enzyme CYP11A1.

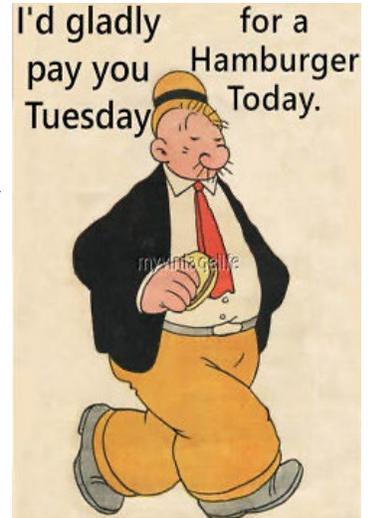


ADRENAL FATIGUE

Adrenal Glands Produce a Variety of Hormones that are Essential to Life. Inadequate Production of One or More of These Hormones as a Result in Adrenal Fatigue



- Fatigue
- Energy Crash
- Depression
- Anxiety
- Poor Sleep
- Lightheadedness
- Frequent Lengthy Infections
- Hair Loss
- Reliance on Caffeine
- Joint Pain
- Brain Fog
- Dark Around Eyes
- Weight Gain
- Craving Carbs
- Craving Salt



Support Your Adrenals



Lower Stress



Eliminate Sugar and Processed Foods



Get Quality Sleep



Cut Excess Coffee

Supplements



Vitamin C

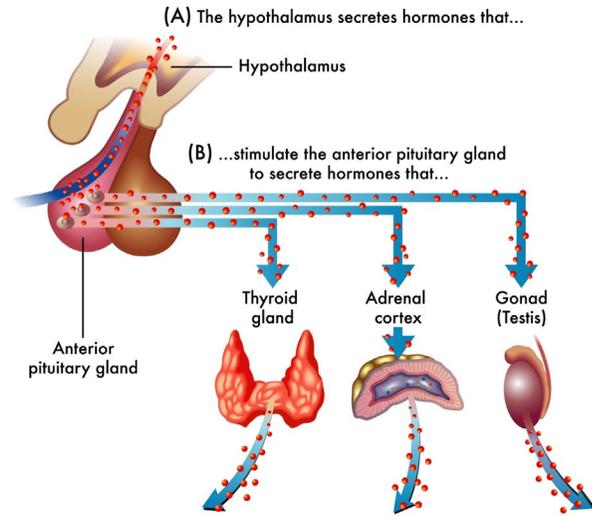


Adaptogenic Herbs



Vitamin B

Hormonal Optimization Requires Energy



Thyroid Function- The Human Metronome

Peak Gonadal Function

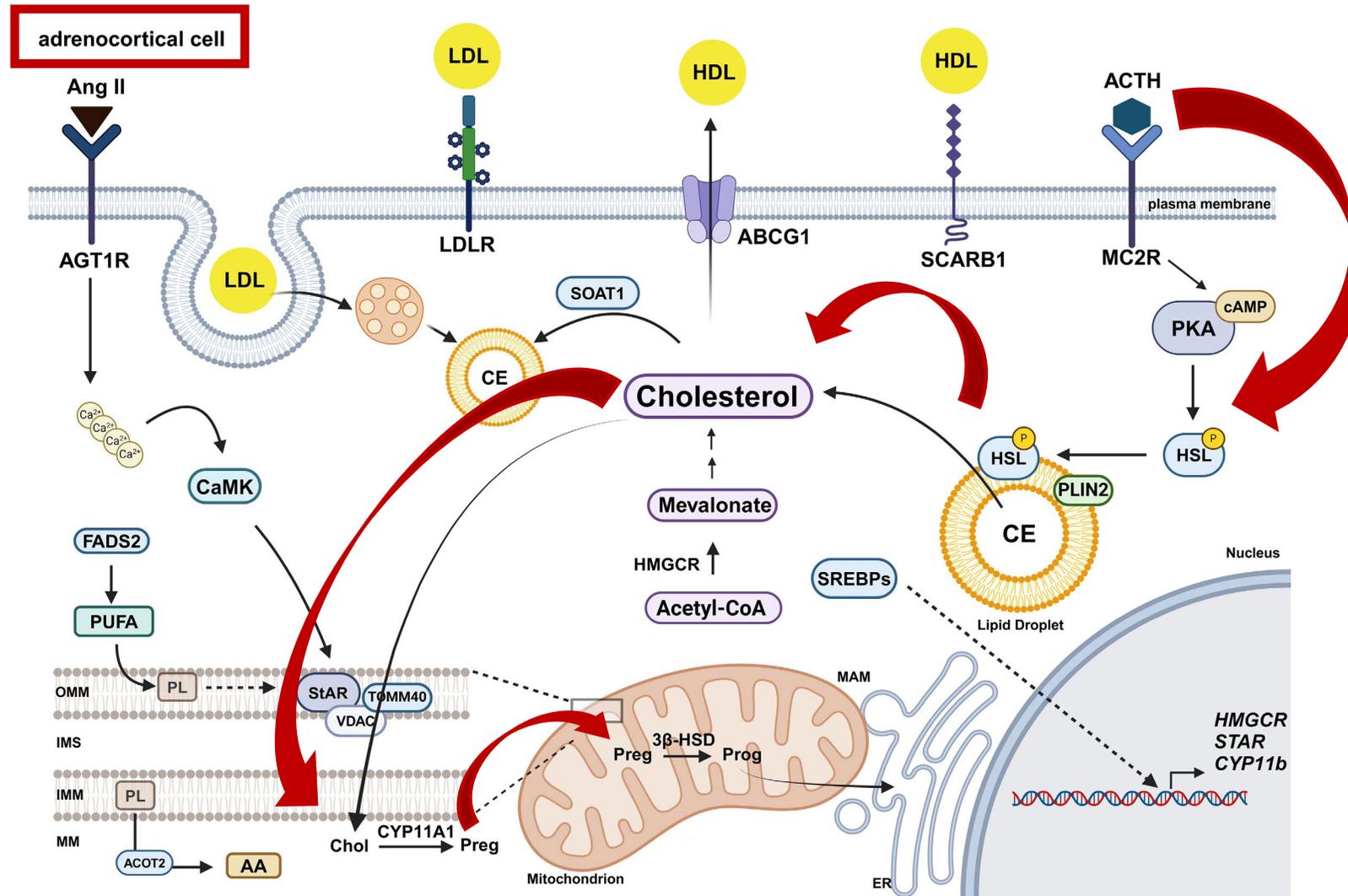
Sleep Restorative Capacity

Adrenal Gland Resilience

Clinical Importance of Global Energy Sufficiency

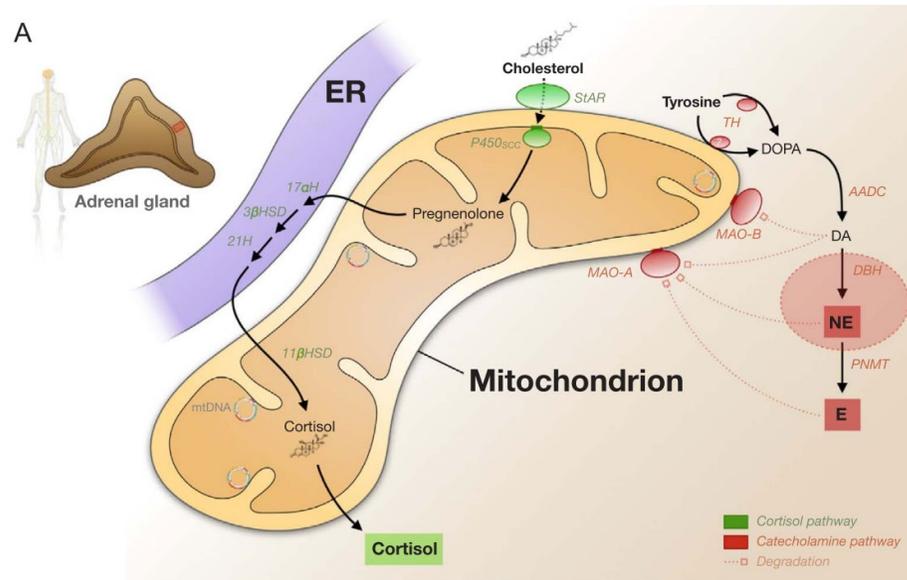


Treat the Mitochondria when Working with Adrenal Patients



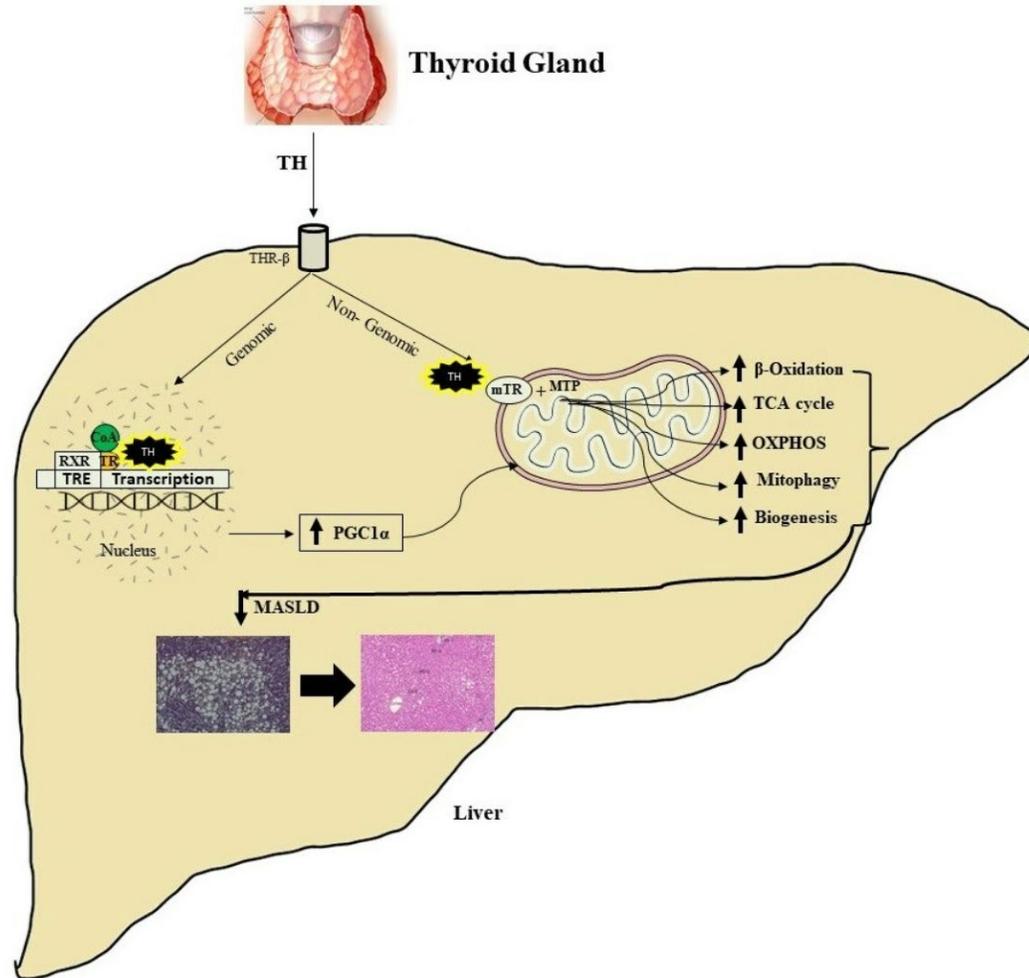
Mitochondrial Sex Hormone Production + Catecholamines

- Patient well being, resilience, adaptive capacity are also modulated via sex hormones---
Testosterone, Estrogen and Progesterone
- Stacking effect- Suboptimal mitochondrial steroidogenesis impacts both stress hormones
(cortisol/cortisone), DHEA and also sex hormones.



The biosynthetic enzymes involved in catecholamine degradation, the monoamine oxidases MAO-A and MAO-B are anchored to the outer mitochondrial membrane.

Dosing Hypothyroid Patients- A Mitochondrial Consideration

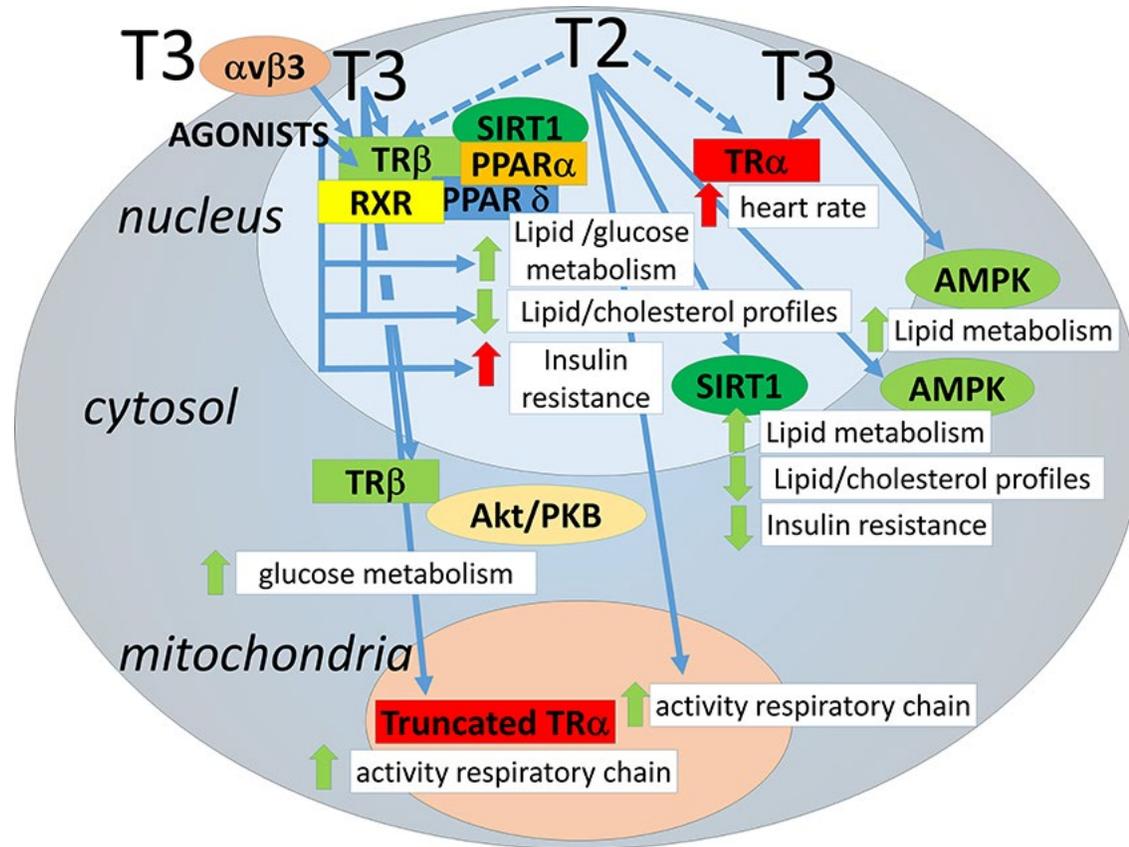


What happens when we treat a hypothyroid patient with T4 or T3?

1. Thyroid stimulates the mitochondria to increase output.
2. Increased cellular demand/BMR
3. Does cells and mitochondria have sufficient requisite substrates?
4. Does the patient with adrenal fatigue have sufficient capacity to handle the workload?



Thyroid Activity of T2 (beyond T3 and T4)



Porcine Thyroid T3/T4 & non-standardized T2



Low Glucocorticosteroids on Immunity and Inflammation

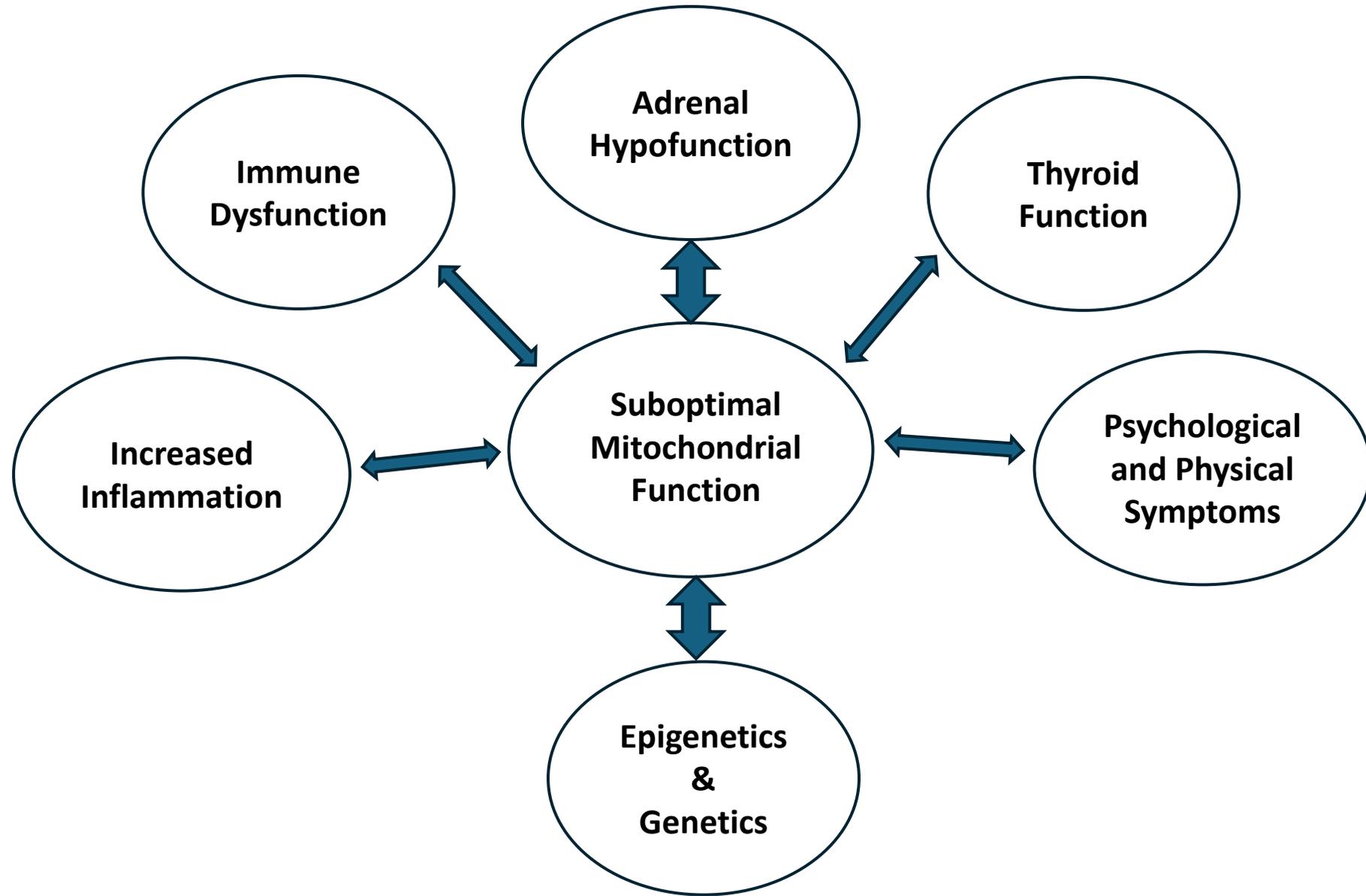
Glucocorticoids are the most potent anti-inflammatory hormones in the body. They **act on the immune system by both suppressing and stimulating pro- and anti-inflammatory mediators.**

Alterations in HPA axis function may have enduring immune effects considering the modulating role of glucocorticoids on the immune system.

A hypocortisolemic stress response, as observed in patients with stress-related disorders, may **result in an overactivity of the immune system in terms of increased inflammatory responses due to impaired suppressive effects of low cortisol levels.**



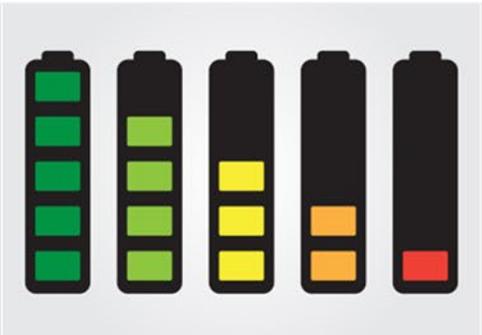
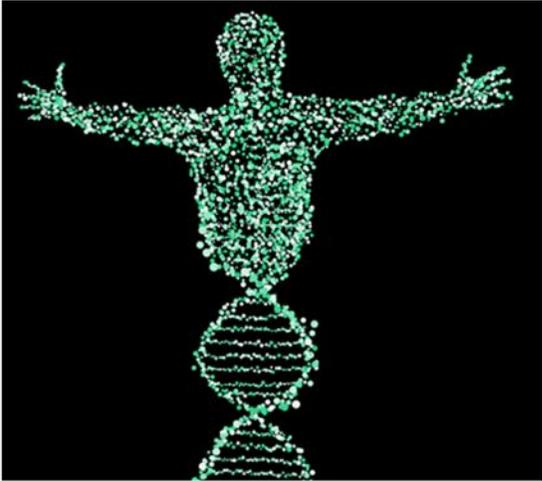
Cumulative Adrenal and Mitochondrial Dysfunction



Functional Medicine 101- Whole Body Repletion

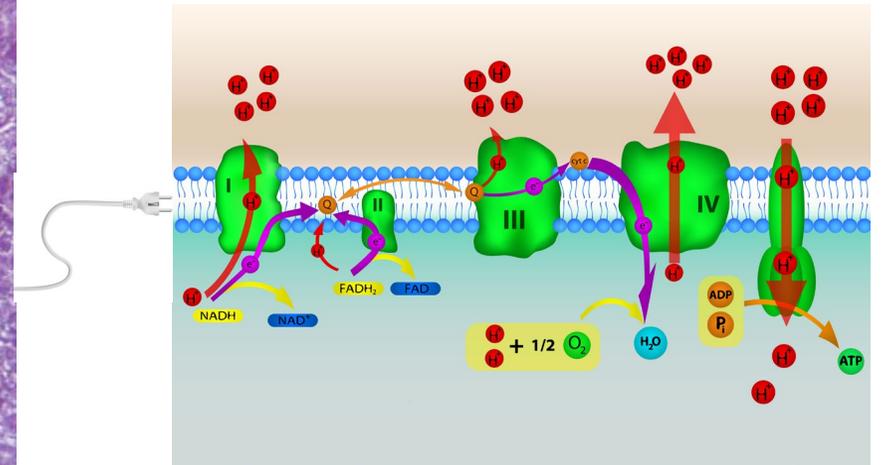
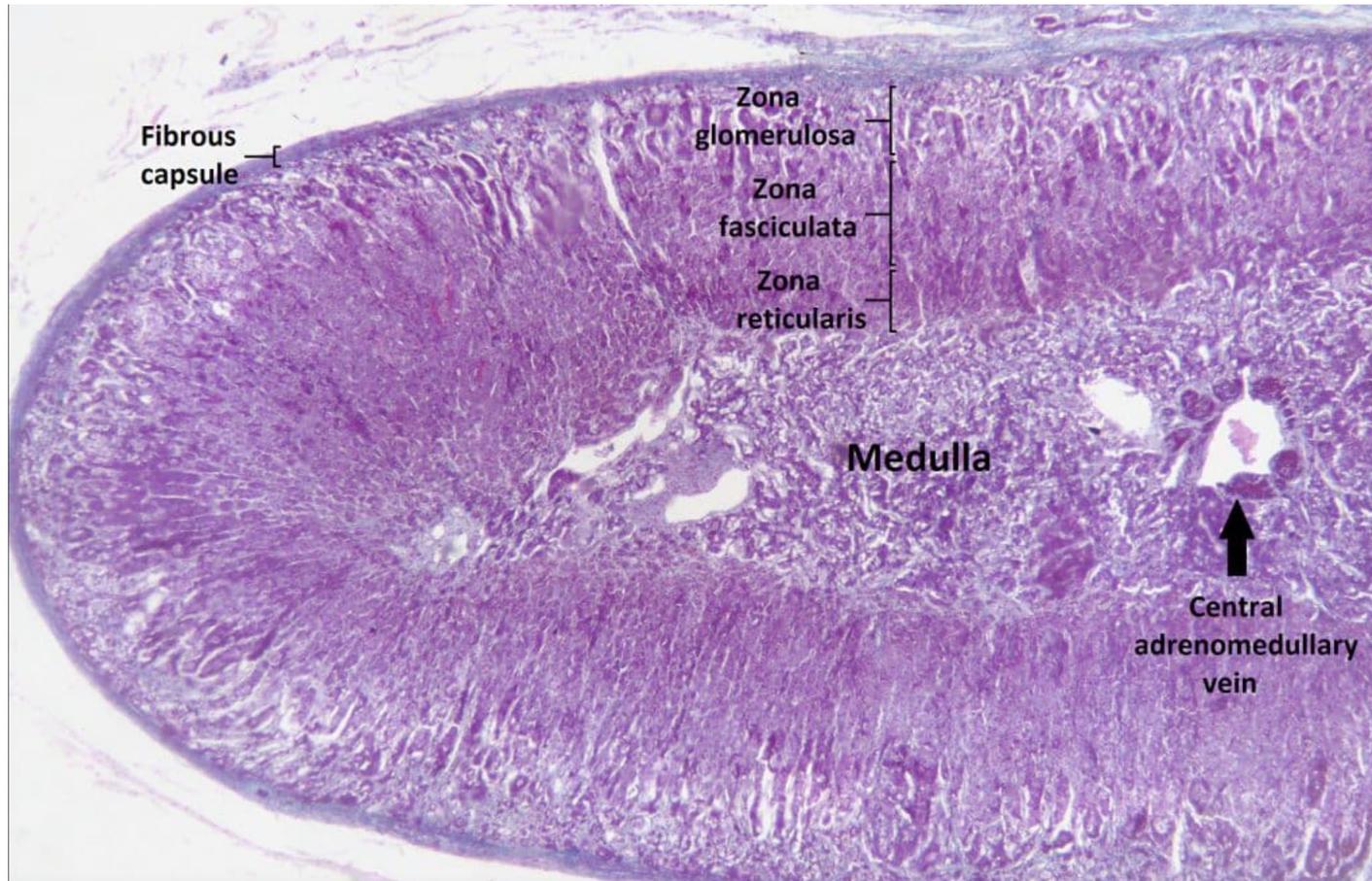


How “Charged Up” are Your Patient’s Cells?



We can't optimize and harness full genetic potential while experiencing an energy deficit

Fueling Adrenal Histology



NAD+ Full Body Therapeutic Considerations



HHS Public Access

Author manuscript

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Therapeutic potential of NAD-boosting molecules: the *in vivo* evidence

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Summary

Nicotinamide adenine dinucleotide (NAD), the cell's hydrogen carrier for redox enzymes, is well known for its role in redox reactions. More recently, it has emerged as a signaling molecule. By modulating NAD⁺ sensing enzymes, it controls hundreds of key processes from energy metabolism to cell survival, rising and falling depending on food intake, exercise and the time of day. NAD⁺ levels steadily decline with age, resulting in altered metabolism and increased disease susceptibility. Restoration of NAD⁺ levels in old or diseased animals can promote health and extend lifespan, prompting a search for safe and efficacious NAD-boosting molecules. **Such molecules hold the promise of increasing the body's resilience, not just to one disease, but to many, thereby extending healthy human lifespan.**

eTOC Blurb

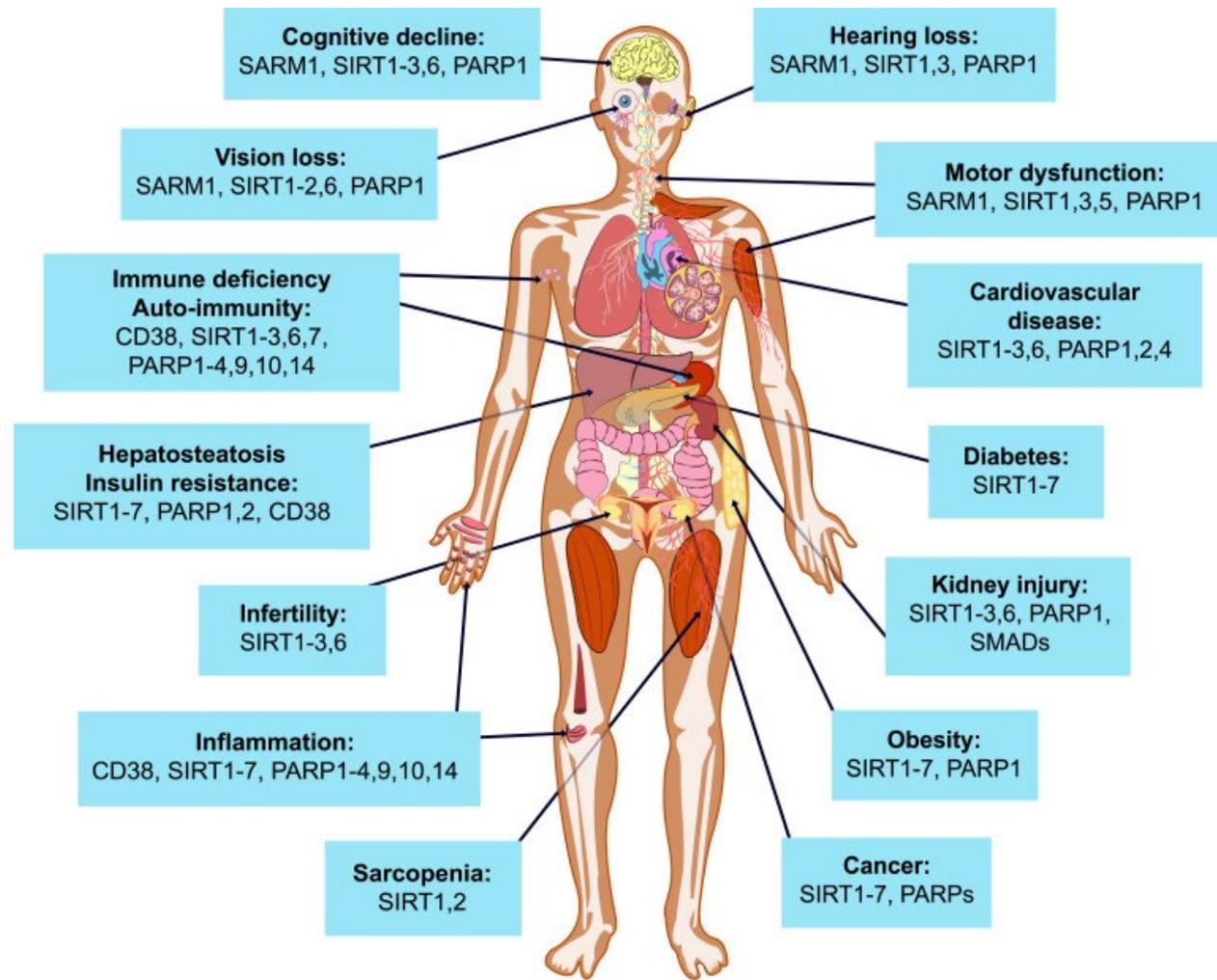
Nicotinamide adenine nucleotide (NAD⁺) has emerged as a key regulator of cellular processes that control the body's response to stress. Rajman et al. discuss NAD boosters, small molecules that raise NAD⁺ levels, which are now considered to be highly promising for the treatment of multiple diseases and the potential extension of human lifespan.

Keywords

aging; epigenetics; sirtuins; chromatin; epigenetics; PARP1; cancer; cardiovascular disease; inflammation; nicotinamide riboside; nicotinamide mononucleotide; CD38; STAC

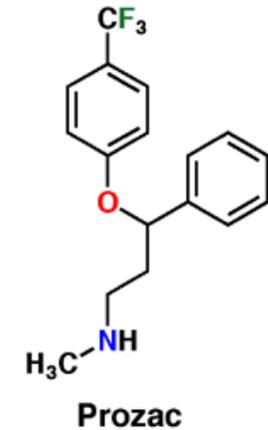
The rise, fall, and rise of NAD

Nicotinamide adenine dinucleotide (NAD) is one of the most important and interesting molecules in the body. It is required for over 500 enzymatic reactions and plays key roles in

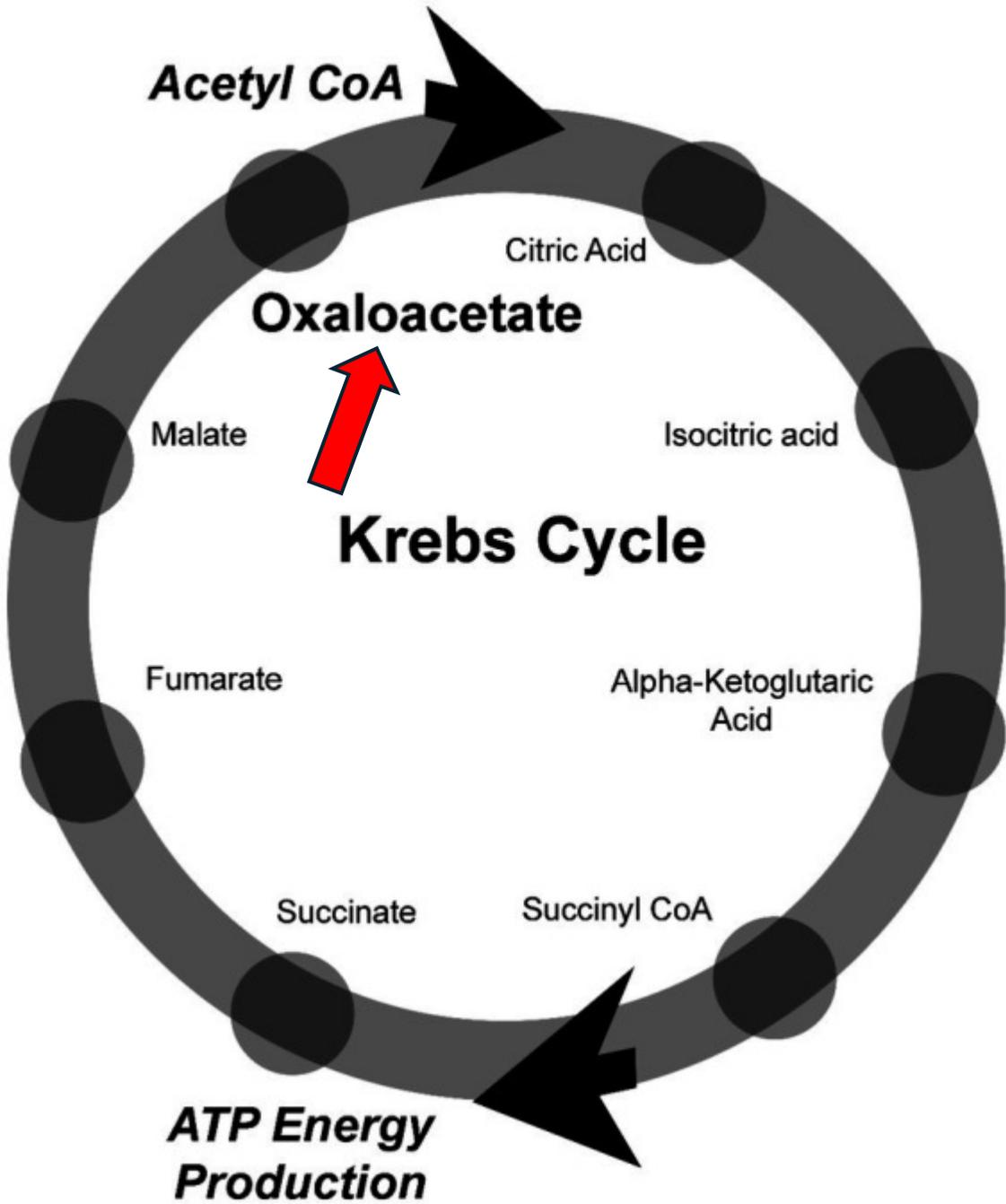


Medications Linked to Mitochondrial Dysfunction

Drug Class	Medications
Alcoholism medications	Disulfiram (Antabuse)
Analgesic and anti-inflammatory	Aspirin, acetaminophen (Tylenol), diclofenac, fenoprofen, indomethacin, naproxen
Anesthetics	Bupivacaine, lidocaine, propofol
Angina medications	Perhexiline, amiodarone
Antiarrhythmic	Amiodarone
Antibiotics	Tetracycline, antimycin A
Antidepressants	Amitriptyline, amoxapine, citalopram, fluoxetine
Antipsychotics	Chlorpromazine, fluphenazine, haloperidol, risperidone, quetiapine, clozapine, olanzapine
Anxiety medications	Alprazolam (Xanax), diazepam (valium)
Barbiturates	Amobarbital, aprobarbital, butabarbital, butalbital, methylphenobarbital, pentobarbital, phenobarbital, primidone, secobarbital, thiobarbital
Cholesterol medications	Statins – atorvastatin, fluvastatin, lovastatin, pitavastatin, pravastatin, rosuvastatin, simvastatin. Bile acids – cholestyramine, clofibrate, ciprofibrate, colestipol, colesevelam
Chemotherapy medications	Mitomycin C, proflomycin, adriamycin (also called doxorubicin and hydroxydaunorubicin and included in the following chemotherapeutic regimens – ABVD, CHOP, and FAC)
Dementia	Tacrine (Cognex), Galantamine (Reminyl)
Diabetes medications	Metformin (Glucophage), troglitazone, rosiglitazone, buformin
HIV/AIDS medications	Retrovir (AZT, ZDV, zidovudine) and several other medications
Epilepsy/Seizure medications	Valproic acid (Depacon, Depakene, Depakene syrup, Depakote, depakote ER, depakote sprinkle, divalproex sodium)
Mood stabilizers	Lithium
Parkinson's disease medications	Tolcapone (Tasmar, Entacapone (COMTan, also in the combination drug Stalevo)



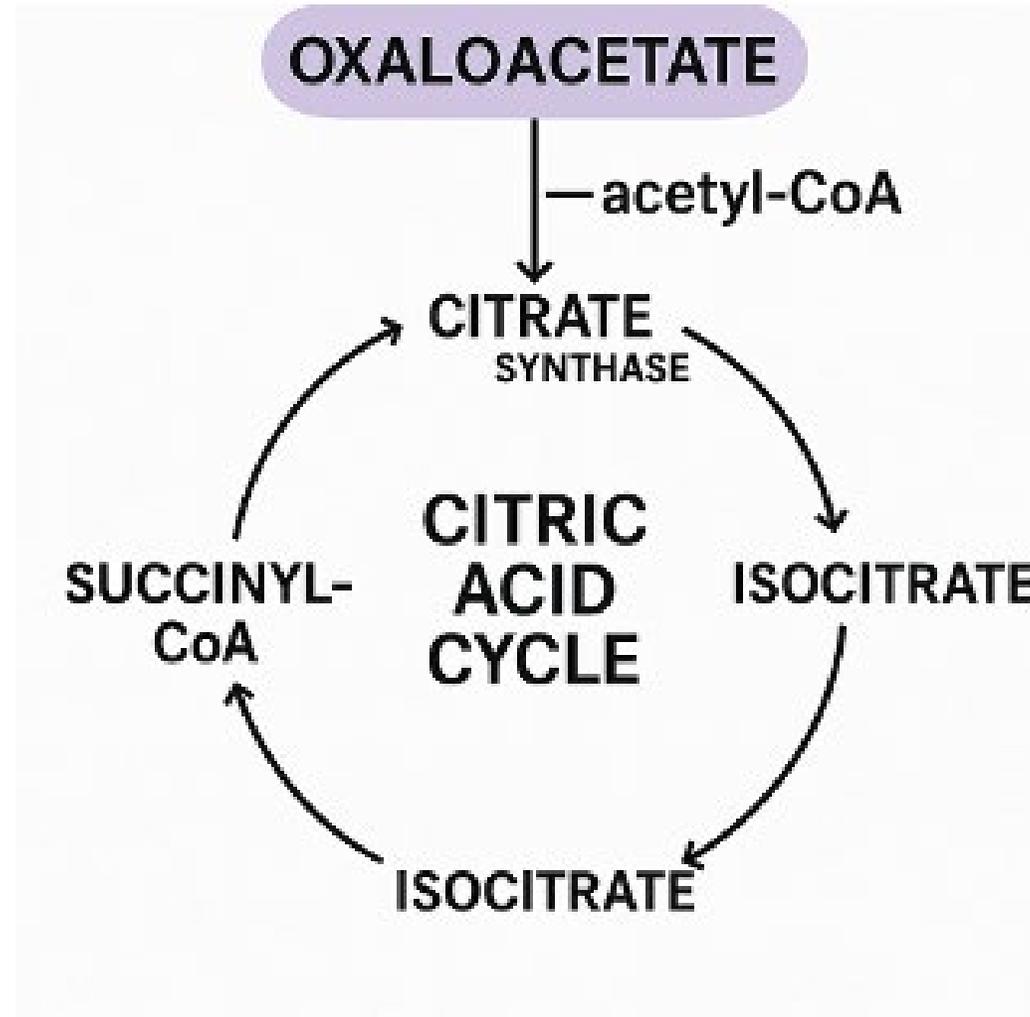
*adapted from Molecular Nutrition & Food Research 2008, 52, 780-788



It is estimated that at least 7% of the more than 700 million confirmed COVID-19 cases reported globally have developed Long COVID.

Symptoms commonly include **fatigue, cognitive impairment, shortness of breath, post-exertional malaise (PEM), and autonomic disturbances**, although the clinical presentation and severity of illness can vary widely.

Oxaloacetate- A Crucial Citric Acid Metabolite



Oxaloacetate Supplementation in Clinical Practice

- Oxaloacetate, a key Krebs cycle intermediate, has gained attention for its ability to **enhance mitochondrial biogenesis, restore NAD⁺/NADH balance, and reduce neuroinflammation.**
- Clinical studies suggest that **stabilized oxaloacetate supplementation can significantly reduce both physical and mental fatigue while improving cognition in patients with CFS and long COVID.**
- Beyond energy metabolism, **oxaloacetate demonstrates antioxidant and metabolic regulatory effects that may help counteract the cellular stress and impaired bioenergetics underlying post-viral syndromes.**

Randomized, Controlled Clinical Trial

- RCT trial of oxaloacetate for symptoms of long COVID, **69 participants were randomized into a group who received either 2,000 mg/day of oxaloacetate or a placebo for 42 days.**
- **At day 21, participants taking oxaloacetate experienced statistically significant improvement in fatigue** as measured by the DePaul Symptom Questionnaire Short Form (DSQ-SF).
- **Total symptom burden improved at day 21.**
- Oxaloacetate supplementation also **significantly improved cognitive performance—the reduction in long COVID symptoms after oxaloacetate supplementation correlated with cognitive improvement.**

Another Study

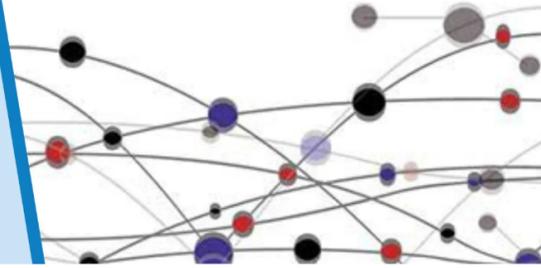
- In another study, researchers investigated the effects of oxaloacetate treatment for mental and physical fatigue caused by either myalgic encephalomyelitis, chronic fatigue syndrome, or long COVID.
- In a non-randomized, controlled, open-label, dose-escalating “proof-of-concept” study, participants were given oxaloacetate or a placebo for six weeks. ME/CFS groups were given **500 mg BID** (n=23), **1000 mg BID** (n=29), or **1000 mg TID** (n=24); Long-COVID groups were given 500 mg BID (n=22) or 1000 mg BID (n=21).

Clinical Findings for Oxaloacetate Oral Intervention

- **At six weeks, 76 ME/CFS patients experienced an average reduction in fatigue.** As measured by the Chalder Fatigue Questionnaire, both physical and mental fatigue were significantly improved over baseline and placebo.
- Fatigue reduction dose dependently increased in ME/CFS patients from:
 - **21.7% for 500 mg BID**
 - **27.6% for 1000 mg BID**
 - **33.3% for 1000 mg TID.**

Fatigue in long COVID patients significantly declined by up to 46.8% in 6 weeks.

CLINICAL TRIAL: REGAIN: A RCT OF OXALOACETATE FOR IMPROVING LONG COVID SYMPTOMS



Clinical Study: REGAIN: a Randomized Controlled Clinical Trial of Oxaloacetate for Improving the Symptoms of Long COVID

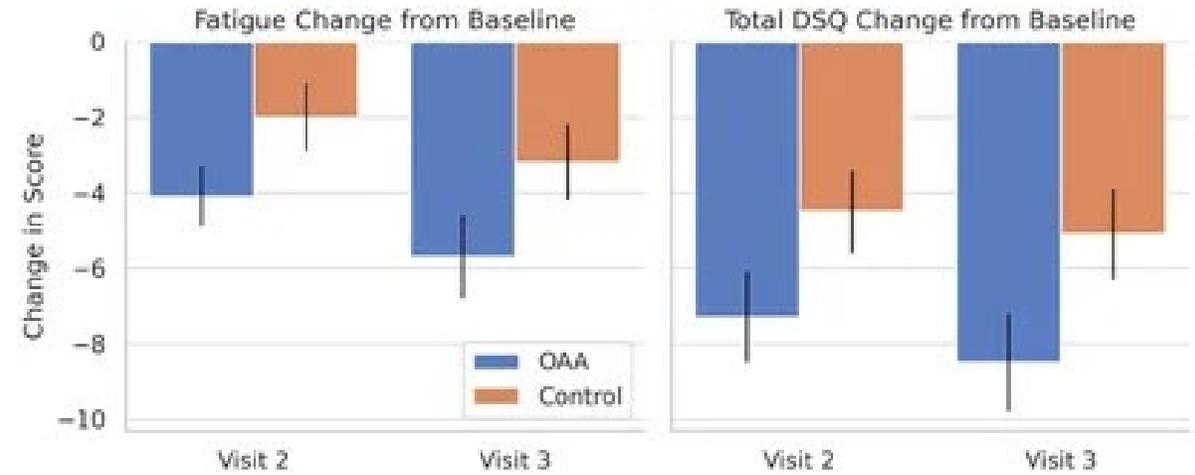
JULY 22, 2025

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Background: Long COVID is characterized by fatigue, cognitive dysfunction, and other persistent symptoms. This randomized, double-blind, controlled trial evaluated the efficacy of oral oxaloacetate (OAA) in improving fatigue and cognitive function in adults with long COVID.

Methods: A total of 69 participants were randomized to receive either 2,000 mg/day of OAA or the control for 42 days. The primary outcome was fatigue reduction, measured by the Chalder Fatigue Questionnaire (CFQ). The secondary and exploratory outcomes included the DePaul Symptom Questionnaire Short Form (DSQ-SF), health-related quality of life (RAND-36), cognitive function (Defense Automated Neurobehavioral Assessment (DANA) Brain Vital), and time upright (UP Time).

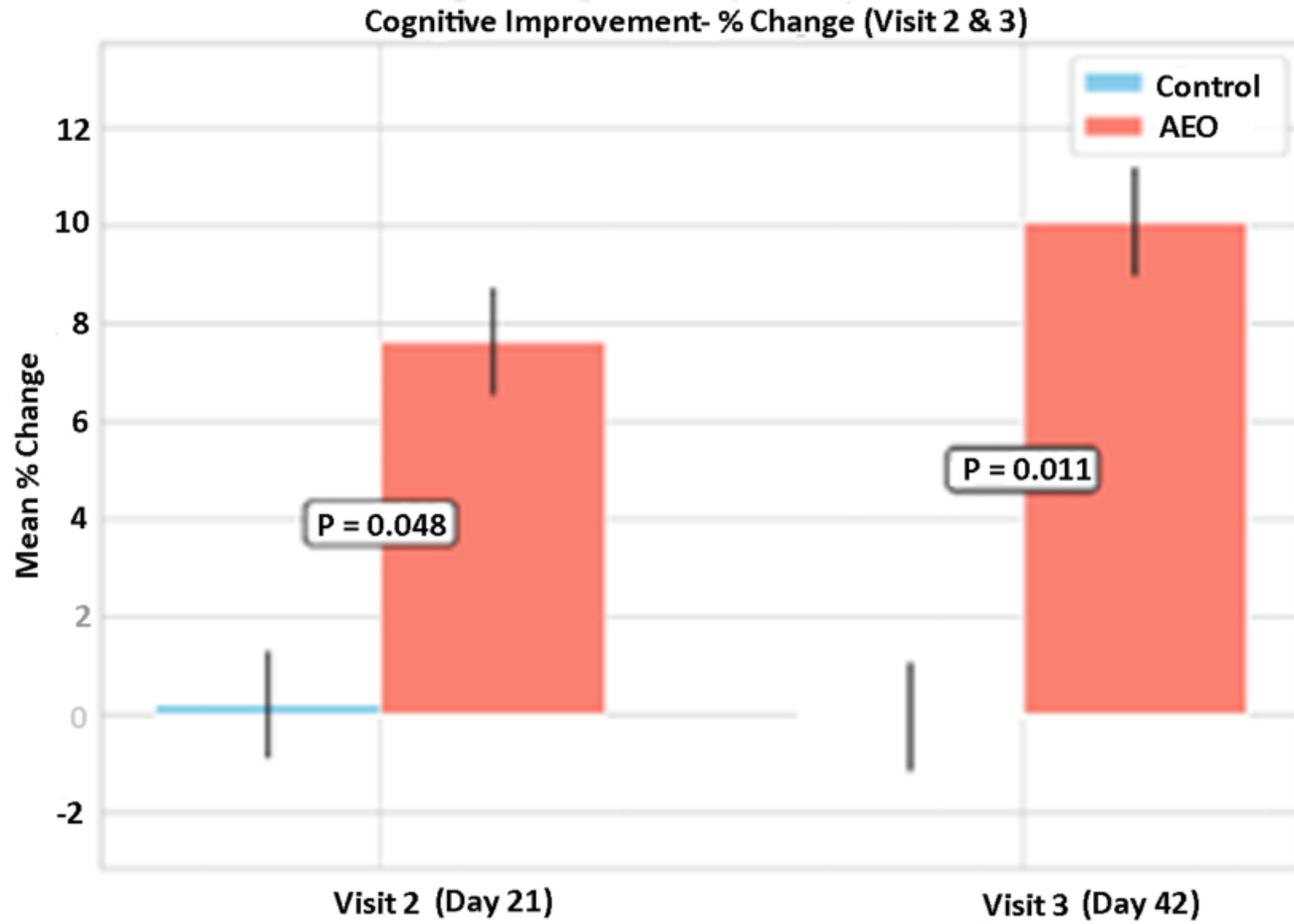
Results: No significant difference in the CFQ-measured fatigue reduction was observed between the groups. However, the OAA group showed significantly greater improvements in the DSQ-SF-measured fatigue and total symptom burden at day 21 of the trial. Cognitive performance improved significantly in the OAA group, with strong correlations between symptom response and cognitive gains. OAA was well tolerated.



There were 69 participants enrolled in this trial, with 35 randomized to the OAA group and 34 randomized to the control group.

Findings support the potential of **OAA to improve symptom burden and cognitive function over a 42-day treatment period relative to control.**

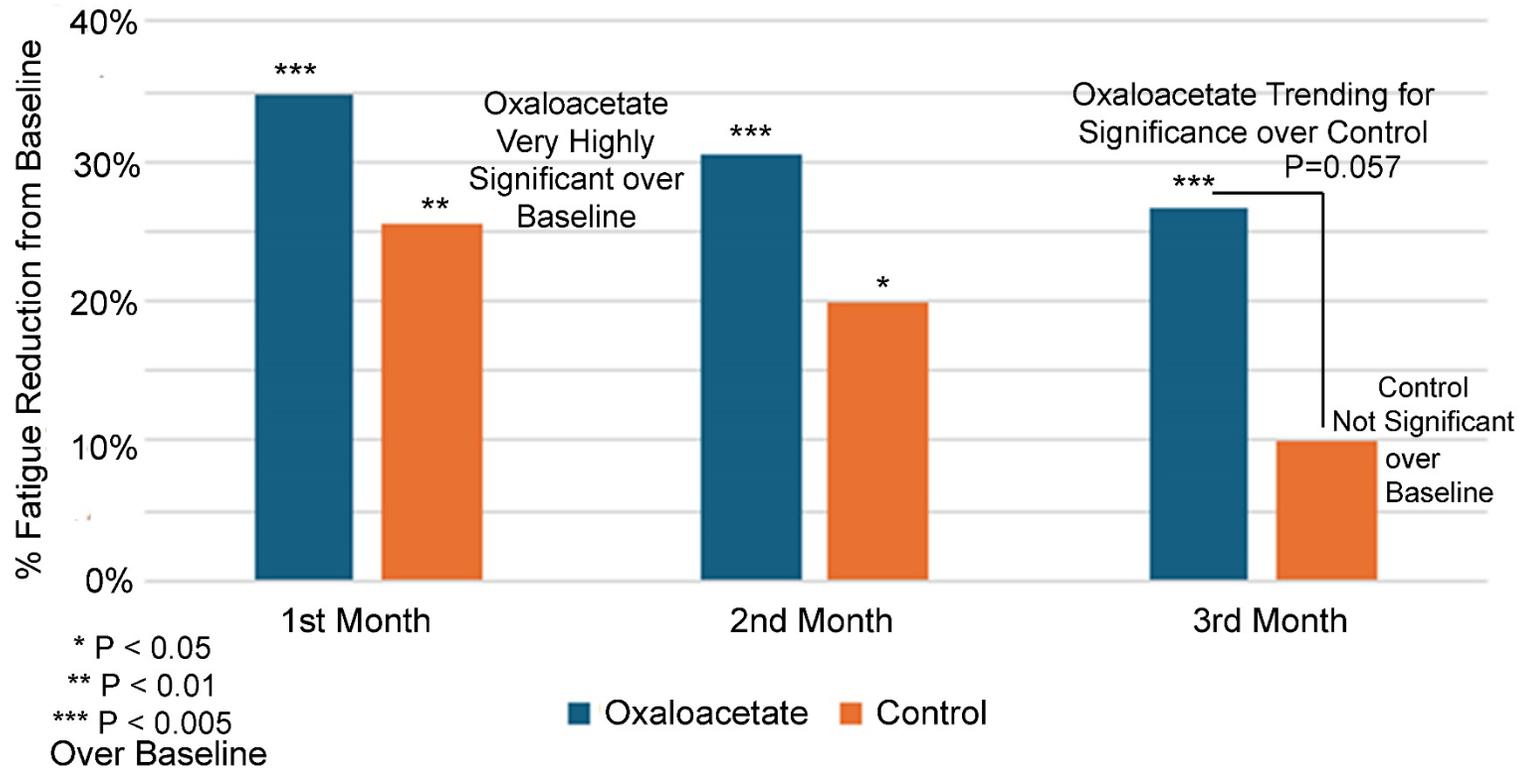
Oxaloacetate – Cognition in Long COVID



- NCT0540237 REGAIN: RCT of AEO for Fatigue in Long COVID
- 69 Patient Trial
- Significant improvement in Cognition within weeks

RCT Oxaloacetate for ME/CFS **NCT05273372**

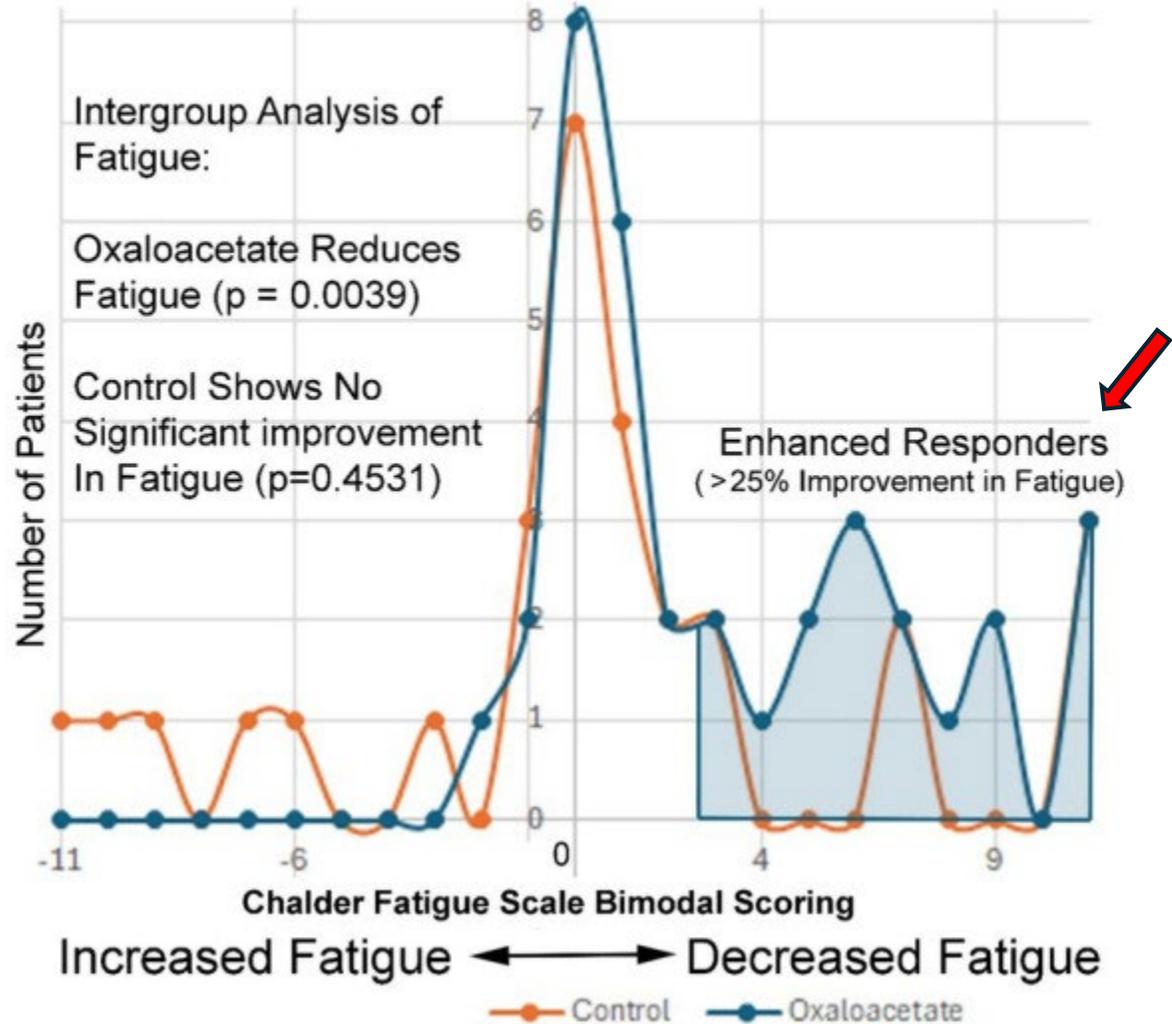
% Reduction In Fatigue From Baseline



Participants that completed the study
N= 64, Bimodal Scoring

Oxaloacetate Group maintains highly significant improvement of 25% to 35% over baseline fatigue levels throughout the study.

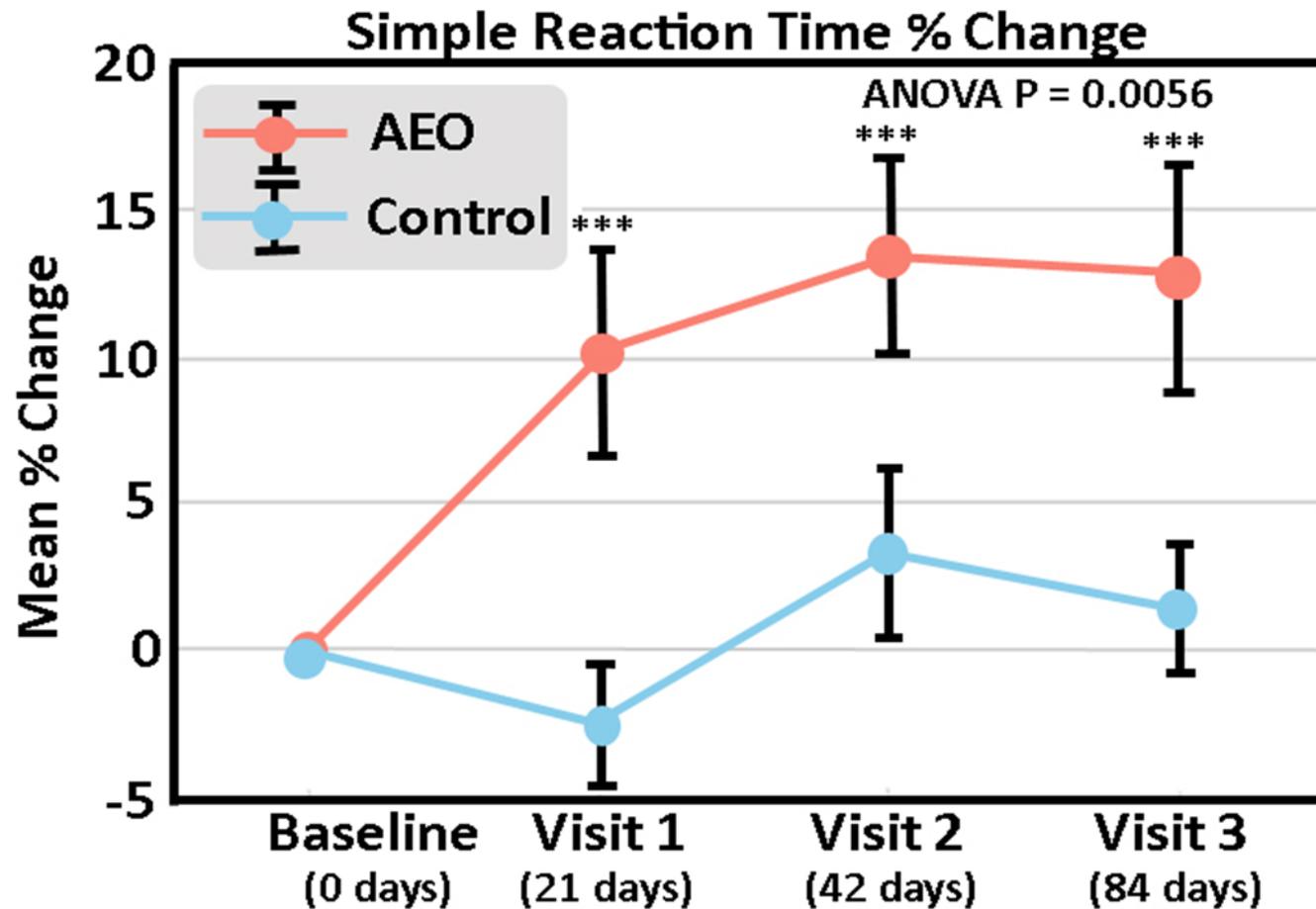
Shift From Day 1 to End of Treatment-- Efficacy Analysis
Chalder Fatigue Score



40.5% patients were enhanced responders with greater than 25% improvement in fatigue

- ✓ **Average improvement of enhanced responders was 63%**
- ✓ Beyond fatigue, they had a significant increase in cognitive function

DANA Brain Vital– Efficacy Analysis



Meta analysis of 50 ME/CFS studies indicated that ME/CFS lowers “Simple Reaction Time”.

<https://pubmed.ncbi.nlm.nih.gov/20047703/>

Oxaloacetate Treatment significantly improves “Simple Reaction Time” over the Control Group, by ANOVA test. (P =0.0056)

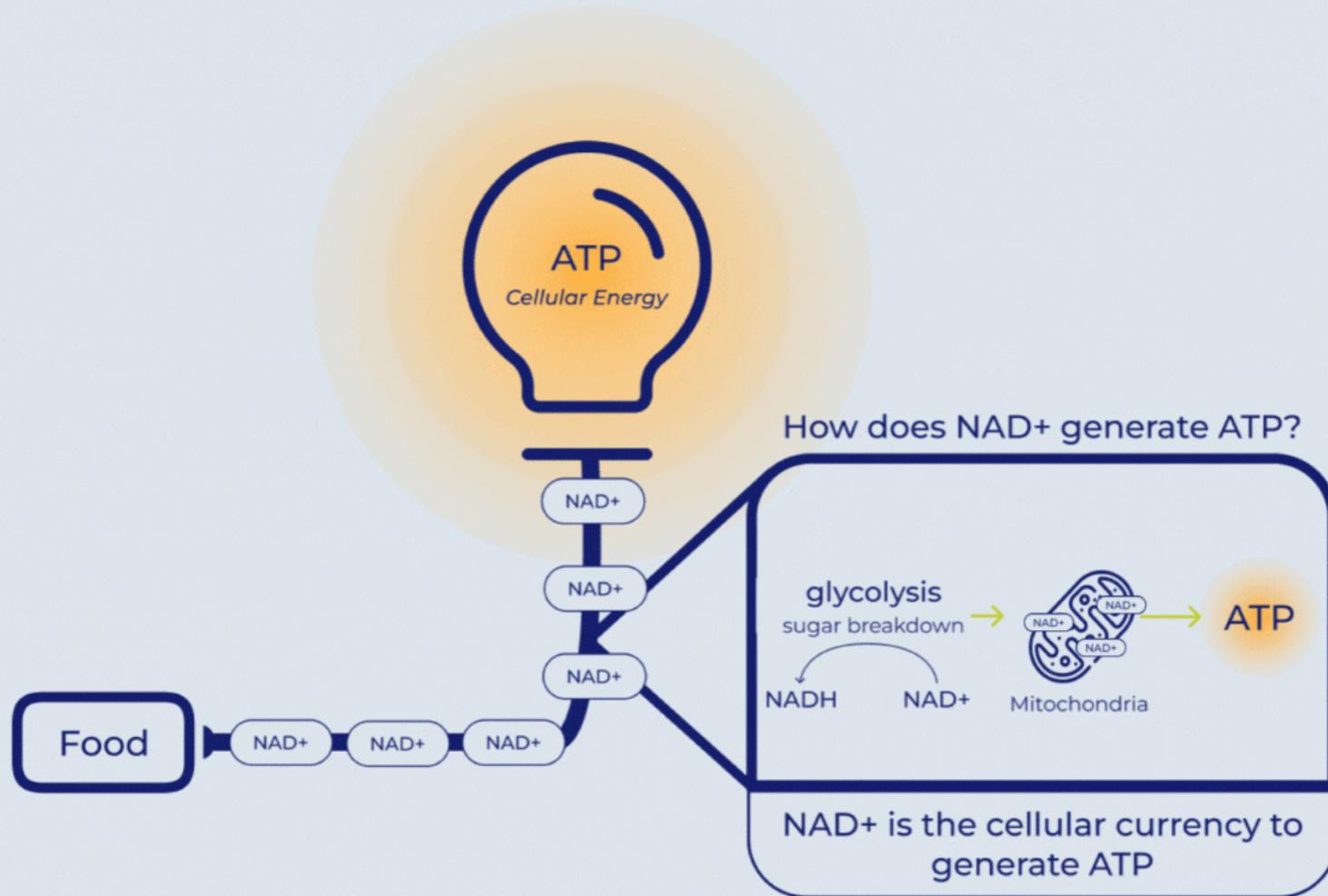
For more information on DANA Brain Vital:
Day H, Yellman B, Hammer S, Rond C, Bell J, Abbaszadeh S, Stoddard G, Unutmaz D, Bateman L, Vernon SD. **Cognitive impairment in post-acute sequelae of COVID-19 and short duration myalgic encephalomyelitis patients is mediated by orthostatic hemodynamic changes.** Front Neurosci. 2023 Jun 26;17:1203514. doi: 10.3389/fnins.2023.1203514. PMID: 37434760; PMCID: PMC10330752.



- Oxaloacetate CFS, the first medical food for the treatment of fatigue in ME/CFS Patients.
- US FDA Allowed Structure/Function Claim:
“Oxaloacetate may help alleviate physical and mental fatigue symptoms Associated with Myalgic Encephalomyelitis/Chronic Fatigue Syndrome (ME/CFS).”

“Medical Food”, which does not require a prescription, but does require medical oversight.

Have We Sufficiently Fueled Our Mitochondria?



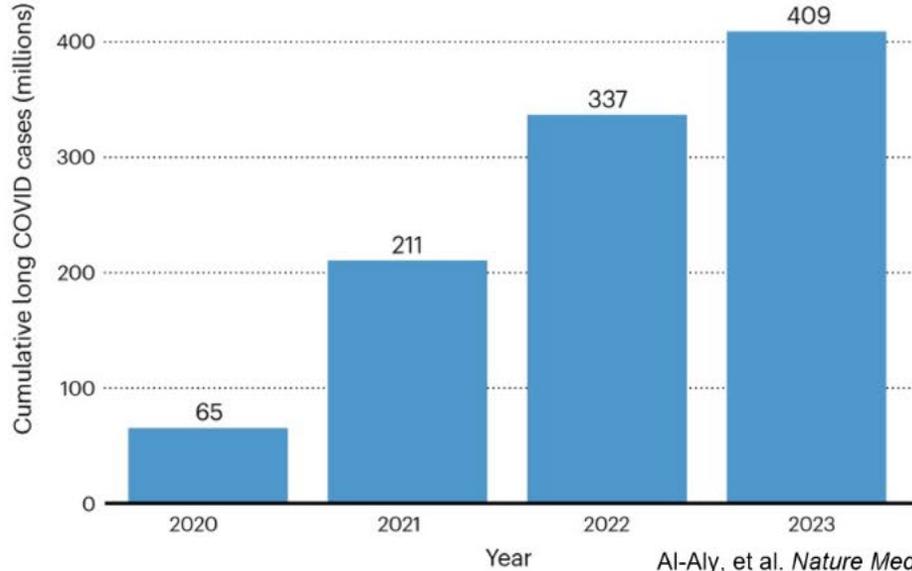
NAD⁺ as a Regulatory Molecule

- “Nicotinamide adenine dinucleotide (NAD⁺) and its metabolites function as critical regulators to maintain physiologic processes, enabling the plastic cells to adapt to environmental changes including nutrient perturbation, genotoxic factors, circadian disorder, infection, inflammation and xenobiotics.”
- During stress, cells overproduce reactive oxygen species (ROS), which damage DNA and activate poly(ADP-ribose) polymerases (PARPs). These enzymes consume NAD⁺ to repair damaged DNA, but excessive PARP activation depletes NAD⁺, impairing mitochondrial function and causing energy deficits.

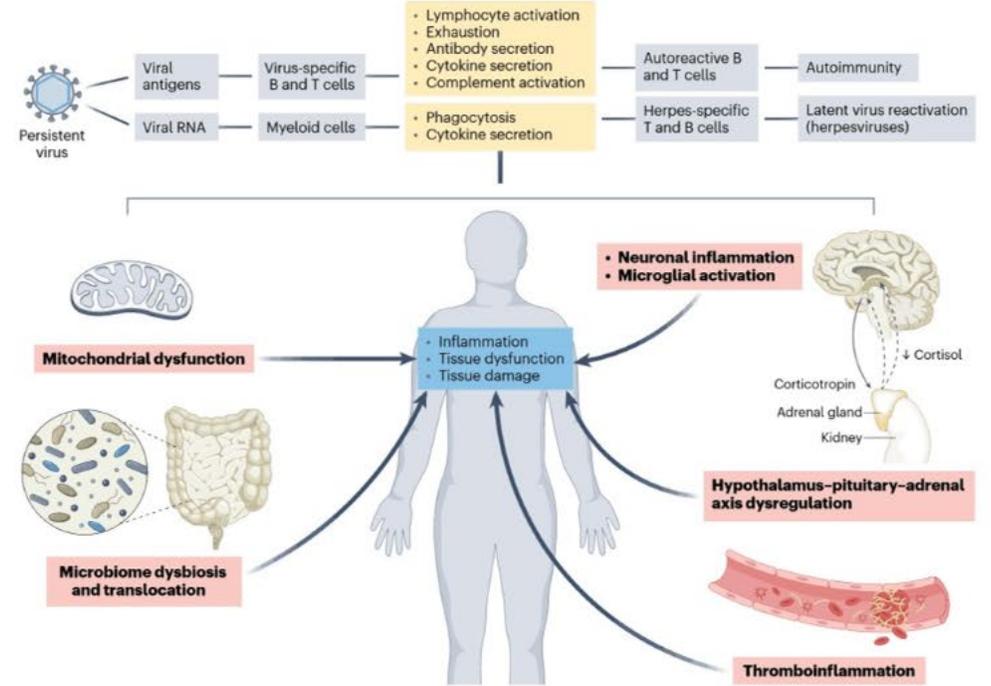
PARPs (poly(ADP-ribose) polymerases) are a family of enzymes that play a crucial role in cellular processes, primarily through DNA repair by recognizing and repairing damaged DNA

Long COVID Harvard Study

Cumulative global incidence of long COVID



Al-Aly, et al. *Nature Medicine* 30, 2148–2164, August, 2024



Completed ⓘ

Clinical Trial of Niagen to Examine Recovery in People With Persistent Cognitive and Physical Symptoms After COVID-19

ClinicalTrials.gov ID ⓘ NCT04809974

Sponsor ⓘ Massachusetts General Hospital

Information provided by ⓘ Edmarie Guzman-Velez, Massachusetts General Hospital (Responsible Party)

Long Covid clinical trial

- RCT, n = 58 Long COVID patients
- 2000 mg/day Niagen for 10 or 20 weeks
- Outcome measures: Cognition, Depression, Sleep quality

COVID and Mitochondrial Involvement

- Mitochondrial dysfunction is a key mediator in similar post-infectious states caused by other diseases.
- There is *potential overlap between the symptoms of long COVID and the well-known negative results of mitochondrial dysfunction.*
- **Research has identified mitochondrial dysfunction in people suffering from long COVID.**
- Scientists have found **impaired mitochondrial respiration and bioenergetics and mitochondria-related gene expression in peripheral blood mononuclear cells (PBMCs) in this group of patients.**

Geroscience. 2024;46(5):5267-5286. | *Int J Mol Sci.* 2023;24(9).

Ann Med. 2025 Dec;57(1):2528167. | *Cell Rep Med.* 2025; 19;6(8):102259.

NPJ Metab Health Dis. 2024;2(1):36.

NAD⁺ Homeostasis

Mechanism	Effect of Stress on NAD ⁺	Physiological Consequence
Oxidative DNA Damage	PARP overactivation depletes NAD ⁺ nature +1	Mitochondrial dysfunction, apoptosis
Chronic Inflammation	CD38 and NADase upregulation nature +1	Reduced NAD ⁺ recycling, metabolic fatigue
Mitochondrial Overload	Hypermetabolism consumes NAD ⁺ faster sciencedirect +1	Decreased ATP, fatigue
Sirtuin Inhibition	NAD ⁺ depletion suppresses SIRT1/3 pmc.ncbi.nlm.nih +1	Weakened stress resilience, aging acceleration

*CD38 is a protein that functions in the body's immune system by regulating calcium signaling, which is critical for immune cell activation and function. It acts as both an enzyme and a receptor, playing roles in T-cell and B-cell activation, leukocyte migration, and inflammation

Mechanisms Linking Stress and NAD⁺ Depletion

- Oxidative and Genotoxic Stress
- During stress, cells overproduce reactive oxygen species (ROS), which damage DNA and activate poly(ADP-ribose) polymerases (PARPs).
- These enzymes consume NAD⁺ to repair damaged DNA, but **excessive PARP activation depletes NAD⁺, impairing mitochondrial function and causing energy deficits.**

Sustained depletion weakens redox balance (NAD⁺/NADH) and increases susceptibility to oxidative injury.

Inflammatory and Neuroendocrine Stress Responses

- **Chronic psychological or physiological stress elevates glucocorticoids and inflammatory cytokines, both of which enhance oxidative processes and NAD⁺ turnover.**
- **Elevated CD38, an NAD-degrading enzyme** induced by inflammation, further accelerates NAD⁺ decline with age and stress.

NAD+ Fueling Over 500 Biochemical Pathways

NAD+ is needed for over 500 biochemical pathways within the body and is essential for ATP production and cellular repair. **In times of increased stress or higher demand for healing, it is of paramount importance to ensure adequate NAD+.**

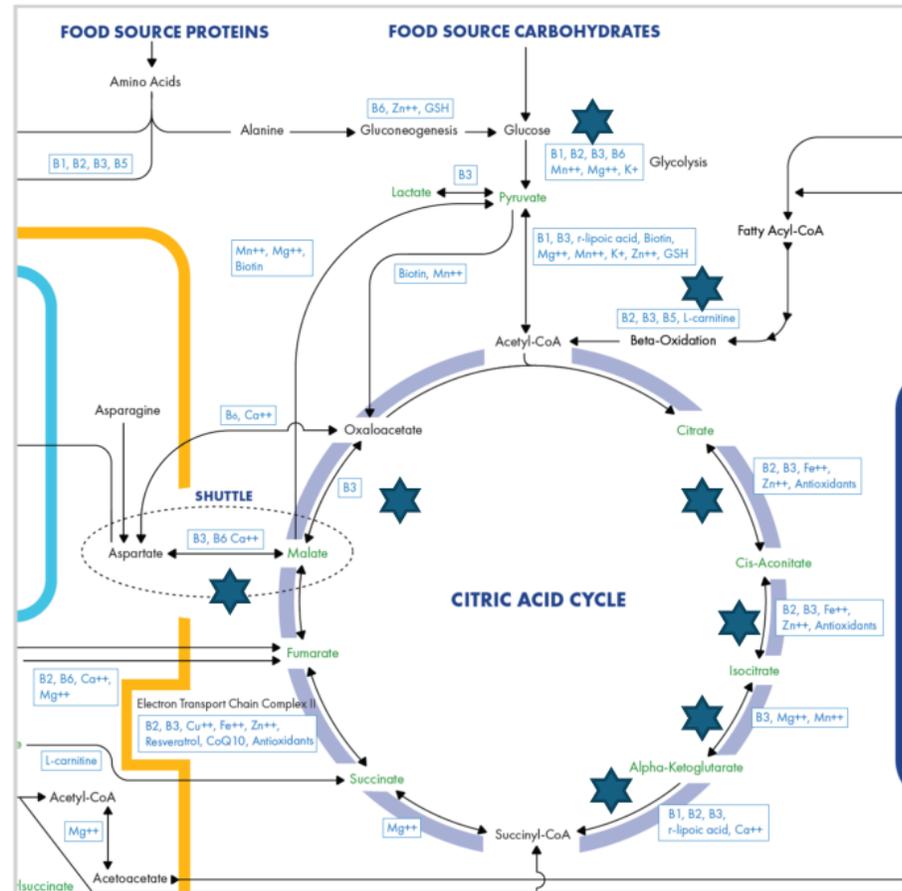


Massudi et al., 2012; Janssens et al., 2022 | Based on analysis of human skin and muscle tissue.

NAD+ decreases over 3-fold by the time we reach young adults.
And another 2.5-fold through middle age.

NAD+ Sufficiency

- Mitochondria, NAD+, and the concept of adrenal fatigue are closely interconnected within the context of energy metabolism and stress physiology.



US BioTek Organic Acids Profile

Provider: _____ Sex: _____ Collected: _____
 Patient: _____ Age: _____ Received: _____
 Accession #: _____ Sample Type: Urine Card Completed: _____

Analyte	Result (µg/mg creatinine)	Reference Range	Population Percentile
Glycolysis			
1. Pyruvate	1.07	< 2.10	56%
2. Lactate	4.21	< 23.10	18%
Citric Acid Cycle			
3. Citrate	69.71	34.30 - 751.30	7%
4. Cis-Aconitate	46.02	< 65.00	81%
5. Isocitrate	29.29	28.00 - 70.00	8%
6. Alpha-Ketoglutarate	6.17	< 26.00	33%
7. Succinate	4.42	< 22.50	33%
8. Fumarate	0.28	< 1.90	16%

“Organic Acids Profile and Environmental Pollutants Interpretations Guide (2021).
 Graphic Courtesy of: US BioTek.

Mitochondrial Metabolic Overload

1. Under heightened energetic demand, mitochondria overutilize NAD⁺ in oxidative phosphorylation. Persistent stress exhausts NAD⁺ stores, reducing ATP production and compromising cellular resilience.
2. Chronic NAD⁺ depletion hinders sirtuin activity (particularly SIRT1 and SIRT3), which normally regulate stress resistance and antioxidant defenses. This creates a vicious cycle, as lower NAD⁺ levels reduce the cell's ability to recover from stress, perpetuating oxidative injury and accelerating biological aging.

Mitochondrial sirtuins in the rat adrenal gland: location within the glands of males and females, hormonal and developmental regulation of gene expressions

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Ludwik K. Malendowicz, Marcin Rucinski

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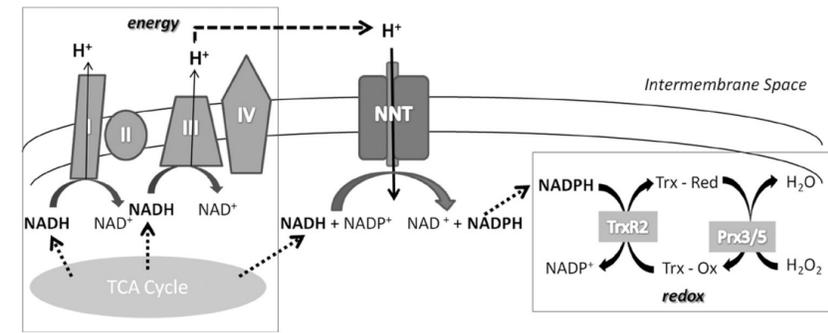
Abstract

Introduction. Sirtuins are NAD dependent class III histone deacetylases. In adrenal cortex mitochondria are able to transform — *via* nicotinamide nucleotide transhydrogenase (NNT) — NAD into NADPH, which is required for steroidogenesis. These findings suggest that sirtuins expressed in mitochondria, Sirt3, Sirt4 and Sirt5, may be associated with adrenal steroidogenesis. Therefore, the purpose of this study was to characterize the expression of mitochondrial sirtuins (Sirt3–5) in individual compartments of rat adrenal cortex, their developmental regulation and to demonstrate whether their expression is dependent on adrenocorticotrophic hormone (ACTH) and Nampt (nicotinamide phosphoribosyltransferase also known as visfatin/PBEF), the rate-limiting enzyme in the regulation of mammalian NAD synthesis.

Material and methods. Studies were performed on rat adrenal glands or on primary culture of rat adrenocortical cells. Expression of mitochondrial sirtuins (Sirt3–5) was evaluated by Affymetrix microarray system or QPCR. The bulk of data were extracted from our earlier experiments which have been reanalyzed in regard to Sirt3–5 mRNAs expression levels and — if necessary — validated by QPCR.

Results. Sirt3–5 were expressed throughout the rat adrenal, with the highest expression level of Sirt5. The level of expression of all sirtuins is higher in the zona glomerulosa (ZG) and zona fasciculata/reticularis (ZF/R) than in the adrenal medulla. Sirt3 and Sirt5 expression levels were similar in adult male and female rats, while Sirt4 expression level was higher in females. As revealed by analysis of the available open database, no significant changes in Sirt3–5 expression levels in whole adrenal glands were observed up to week 104 of life of both male and female rats. Moreover, 60 min after intraperitoneal ACTH injection the expression level of Sirt3 in the entire gland was elevated while Sirt5 expression level lowered. On the other hand, chronic ACTH infusion (48 h) did not change expression of studied sirtuins. In cultured cells, ACTH greatly increased the expression levels of the Sirt4 and Sirt5. In cultured cells, Fk866 — a highly specific competitive inhibitor of Nampt — reduced expression level of Sirt5 only. In enucleation-induced regenerating rat adrenal, the expression levels of all studied sirtuins were significantly reduced in relation to the control group. Finally, in primary rat adrenal culture the FCS depletion elevates the Sirt3 and Sirt4 expression levels and downregulates Sirt5 expression.

Conclusions. Sirt3–5 are expressed throughout the rat adrenal, with the highest expression levels in adrenal cortex. Performed experiments (ACTH stimulation, FCS depletion, regeneration) suggest that in the adrenal cortex, the mitochondrial Sirt5 is the primary mitochondrial sirtuin involved in regulating the biological activity of adrenocortical cells. Our results also suggest that normal levels of intracellular Nampt (iNampt) enzymatic activity are required to maintain normal (control) levels of Sirt5 mRNA in cultured cells. (*Folia Histochemica et Cytobiologica* 2017, Vol. 55, No. 4, 190–202)



Abstract

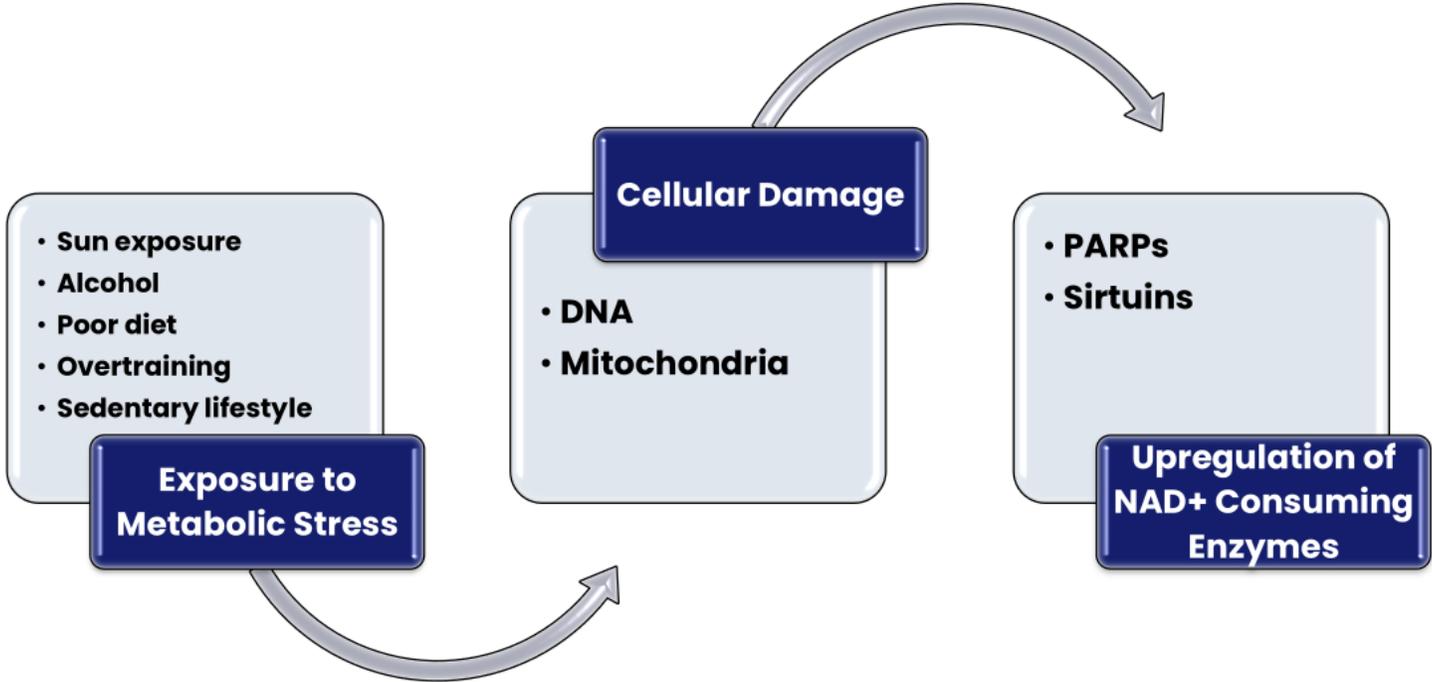
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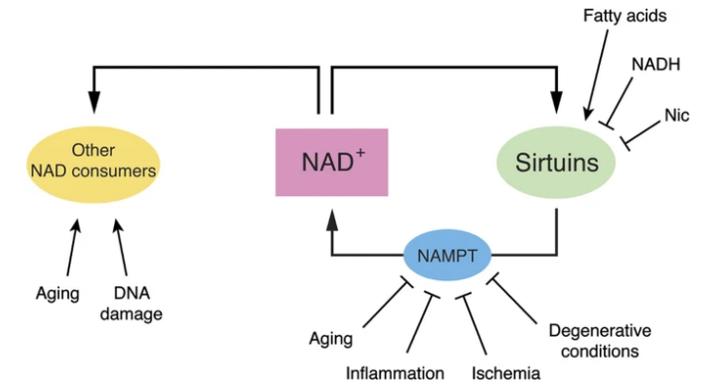
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NAD+ Consumed to Repair Damage



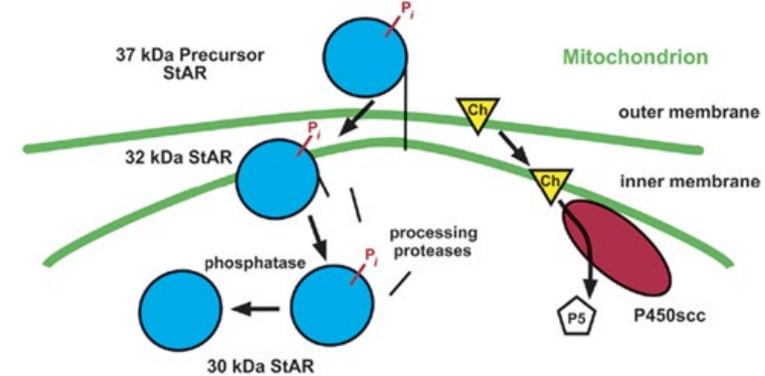
↑ stressors → ↑ NAD+ consumption → ↓ NAD+ levels → ↓ cell function

Mitochondrial Sirtuins in the Rat Adrenal Gland



- **Sirt3-5 are expressed throughout the rat adrenal**, with the highest expression levels in adrenal cortex.
- The mitochondrial Sirt5 is the primary mitochondrial sirtuin involved in regulating the biological activity of adrenocortical cells.
- The availability of NAD in adrenocortical cells may determine normal steroidogenesis. This suggestion is supported by recent findings demonstrating that NNT mutations can be the cause of primary adrenal insufficiency (combined mineralocorticoid and glucocorticoid deficiency).

NAD or NAD/NADH ratio

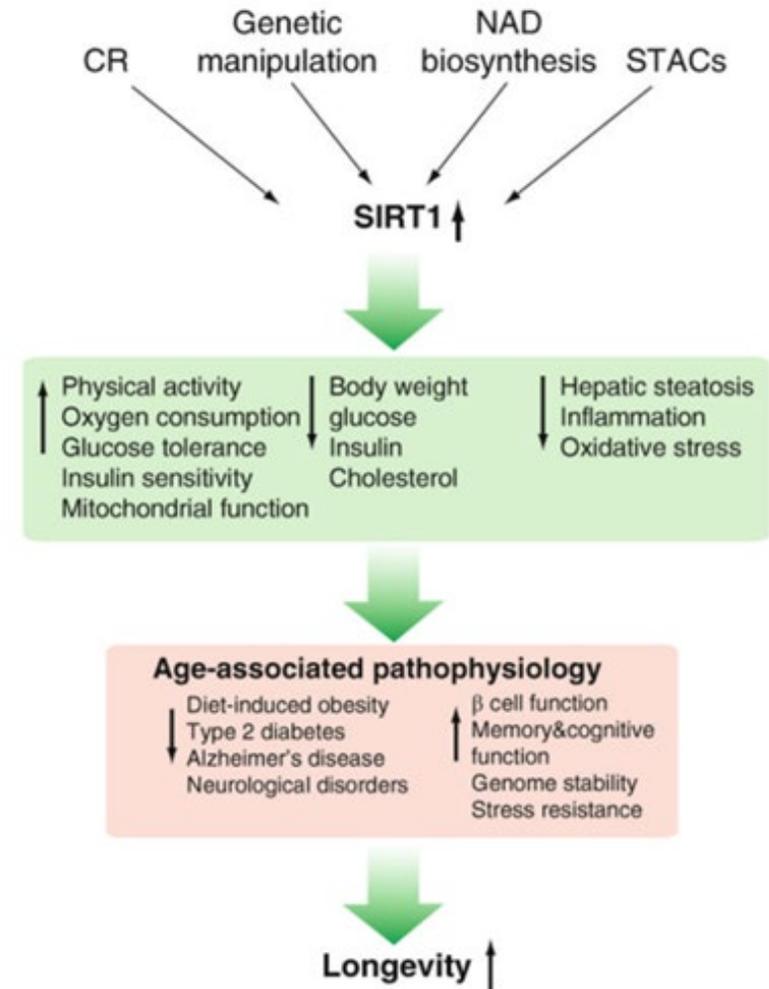


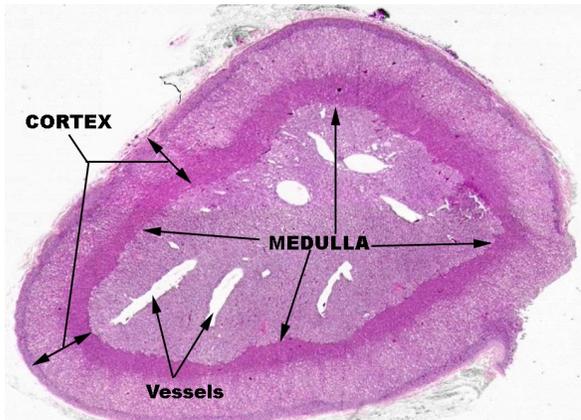
- It has been suggested that **all sirtuins function as sensors of the cellular energy status represented by NAD or NAD/NADH ratio.**
- Adrenal gonadal, placental and brain mitochondria contain **several steroidogenic enzymes, notably the cholesterol side chain cleavage enzyme, P450scc, which is the enzymatic rate-limiting step in steroidogenesis which determines cellular steroidogenic capacity.**
- The **access of cholesterol to this enzyme system is both rate-limiting and the site of acute regulation via the steroidogenic acute regulatory protein (StAR)** which interacts with a complex multi-component 'transduceosome' on the outer mitochondrial membrane (OMM).

Fueling Sirtuins to Support Homeostasis

The enzymatic activity and localization of mammalian sirtuins

	<u>Enzymatic activity</u>		<u>Localization</u>
	<u>Deacetylase</u>	<u>ADP-ribosyltransferase</u>	
<i>Sirt1</i>	✓		Nucleus, cytoplasm
<i>Sirt2</i>	✓		Cytoplasm, nucleus
<i>Sirt3</i>	✓		Mitochondria
<i>Sirt4</i>		✓	Mitochondria
<i>Sirt5</i>	✓		Mitochondria
<i>Sirt6</i>	✓	✓	Nucleus
<i>Sirt7</i>	✓		Nucleolus





Adrenal Mitochondria and Steroidogenesis: From Individual Proteins to Functional Protein Assemblies

In this paper, we discuss this functionalization, beginning with the tissue zonation of the adrenal cortex and how this impacts steroidogenic output. We then discuss the cellular biology of steroidogenesis, placing special emphasis on the mitochondria. Mitochondria are classically known as the “powerhouses of the cell” for their central role in respiratory adenosine triphosphate synthesis, and attention is given to mitochondrial electron transport, in both the context of mitochondrial respiration and mitochondrial steroid metabolism. Building on work demonstrating functional assembly of large protein complexes in respiration, we further review research demonstrating a role for multimeric protein complexes in mitochondrial cholesterol transport, steroidogenesis, and mitochondria–endoplasmic reticulum contact. We aim to highlight with this review the shift in steroidogenic cell biology from a focus on the actions of individual proteins in isolation to the actions of protein assemblies working together to execute cellular functions.

- Nicotinamide Riboside
- CoQ10
- PQQ
- Creatine
- Adrenal Support
 - DHEA
 - Pregnenolone
 - Adaptogenic Botanicals
 - Vitamin C
 - Pantothenic Acid

Metabolic Stressors that Deplete NAD+



Courtesy of Niagen Bioscience

Niagen.
BIOSCIENCE

State of the Science

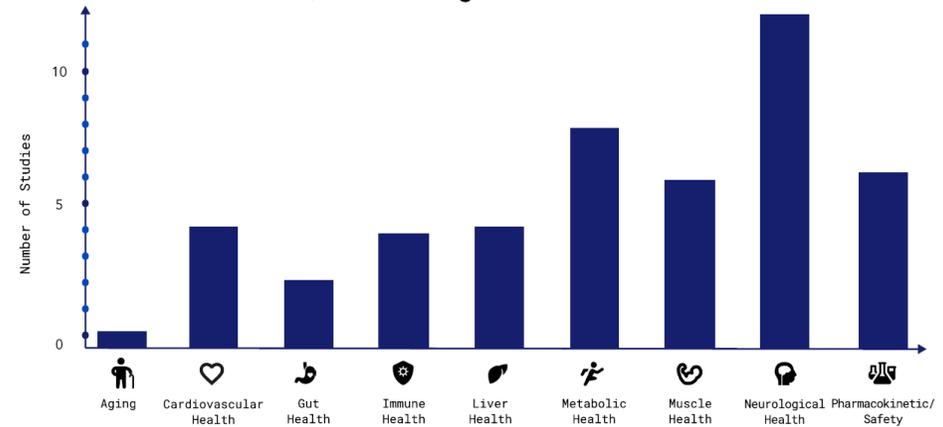
Clinical Research on Niagen®



TRU NIAGEN®

Clinical Research on Niagen®

Peer-Reviewed, Published Niagen® Clinical Studies Per Health Area



2 years

is the longest duration of supplementation

Presterud et al., 2023

140 participants

is the largest population studied in a clinical trial

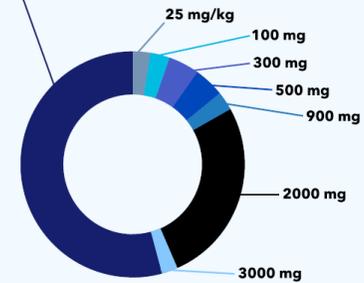
Conze et al., 2019

3000 mg

is the highest dose with established safety

Berven et al., 2023

1000 mg is the most extensively researched daily dose.



92% of Niagen® published, peer-reviewed studies were conducted independently.

35 studies were conducted independently

3 studies were funded by ChromaDex



Courtesy of Niagen Bioscience

TRU NIAGEN®

Fueling the 37 Trillion Cells of Our Patients

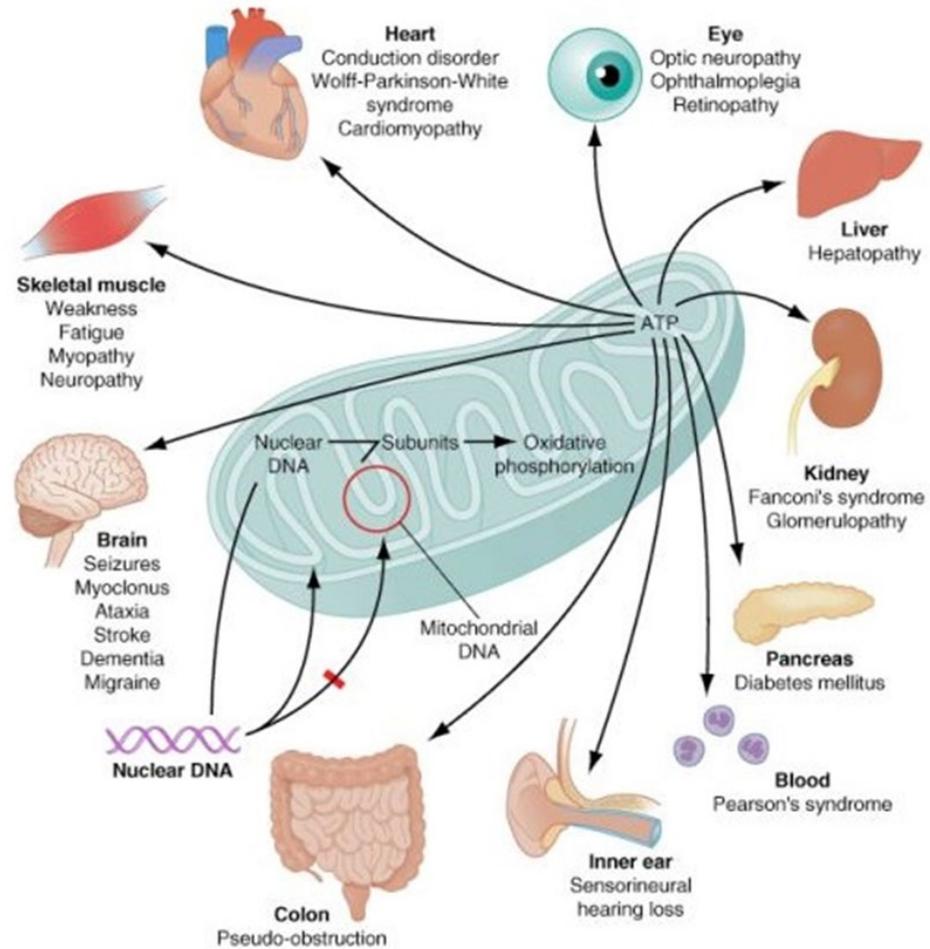


Table 2. A Select Sampling of Functional Medicine Approaches to Long COVID*

Agent	Mechanism of Action
Bromelain	Proteolytic actions ³
Nattokinase	Proteolytic actions ³
Curcumin	Modulates inflammation, antiviral actions ³
N-Acetylcysteine (NAC)	Dissolves spike protein through the destruction of disulfide bonds and prevents binding at ACE2, works together with bromelain ⁴⁻⁶
Glutathione	Disrupts spike protein disulfide bonds ⁶
Quercetin	Binds and inhibits spike protein ⁷
Apigenin	Binds and inhibits spike protein ⁷
Nicotine	Disrupts glycosylation on spike protein and blocks possible spike protein-nicotinic cholinergic receptor interaction ^{8,9}
Emodin	Blocks the spike protein-ACE2 interaction ¹⁰
Fisetin	Binds and inhibits spike protein ¹¹
Rutin	Binds and inhibits spike protein ¹²
Silymarin	Binds and inhibits spike protein ¹³
Oxaloacetate	Rejuvenates the mitochondria to reduce fatigue
NAD+ Support	 Adress Physiological Demand & Mitochondria

*The following list is not intended to be exhaustive, rather a sampling of potential clinical considerations to add to your existing protocols, with individual consideration to each patient's unique biochemistry and physiology.

My Clinical Approach to Adrenal Fatigue

1. **Minimize Stress and Stress Response** (L-Theanine and Relora®)
2. **Restorative Sleep** (rule out sleep apnea, snoring partner, dark room, early to bed, avoid blue light exposure 2 hours prior to bed)
3. **Daily relaxing exercise and Forest Bathing** (Shinrin-yoku)
- ➡ 4. **NAD⁺ Support** (Nicotinamide Riboside (Niagen®), Diet, Exercise)
- ➡ 5. **Mitochondria Support-** CoQ10, Carnitine, Niagen® and Target Nutrients
6. **Adaptogenic Botanicals-** Maca, Rhodiola, Ashwagandha, Ginseng, etc.
7. **Nutrient Sufficiency-** Vitamin and Nutrient Testing
8. **Vagal Nerve Tone-** Focus on balancing sympathetic and parasympathetic nervous system
9. **Adrenal Testing**
 - a) Cortisol 4x, DHEA-sulfate, Pregnenolone
 - b) Neurotransmitter Testing (Dopamine, Epinephrine, Norepinephrine, GABA, Serotonin, etc.)
 - c) Melatonin Levels



Whole Body Wellness while Helping with Mitochondria



TIME FOR QUESTION.....Thank you!

Resources References:

Microplastic Lab
RSlabs.health
PW 224466

Pro.TruNiagen.com



OxaloacetateCFS.com

Wholesale Code: WH-OAA-199

It's Time To Start Living Again

Is ME/CFS or Long COVID robbing you of the life you deserve?

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Oxaloacetate CFS 500 mg is a medical food *clinically proven to lower fatigue* in patients with Long COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome.

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Oxaloacetate CFS
A Clinically Proven Medical Food
For Chronic Fatigue Syndrome