MITODICURE

A COMPREHENSIVE DISEASE CONCEPT BASED ON EXERCISE INTOLERANCE AND A DERIVED THERAPEUTIC STRATEGY

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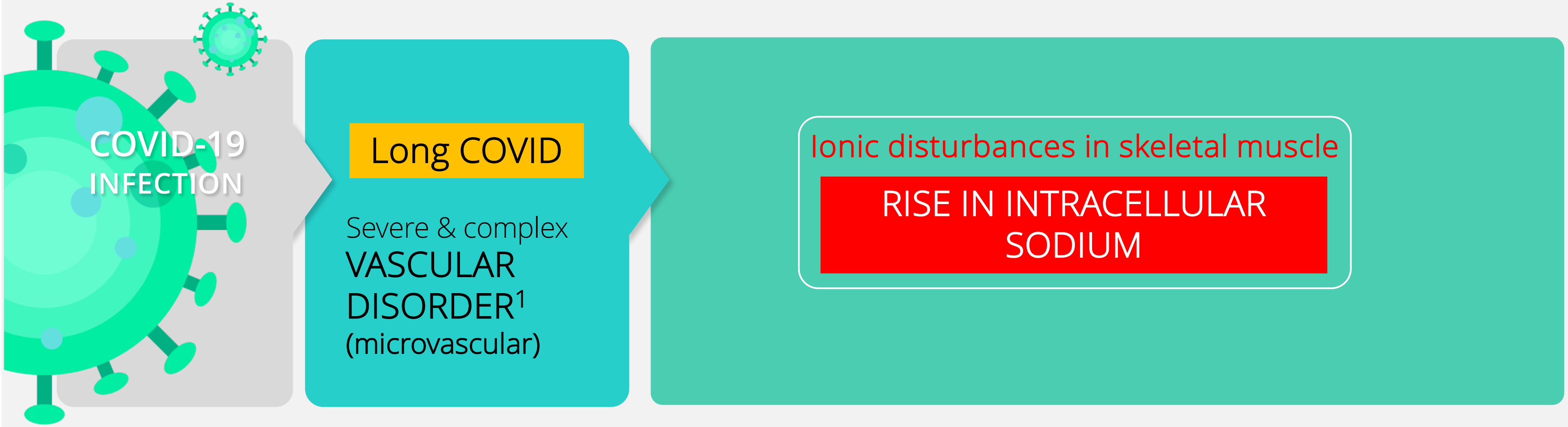
DISTURBED PERFUSION IN POST-COVID-SYNDROME LEADS TO AN INCREASE IN INTRACELLULAR SODIUM IN THE SKELETAL MUSCLE CELLS DURING MUSCLE WORK

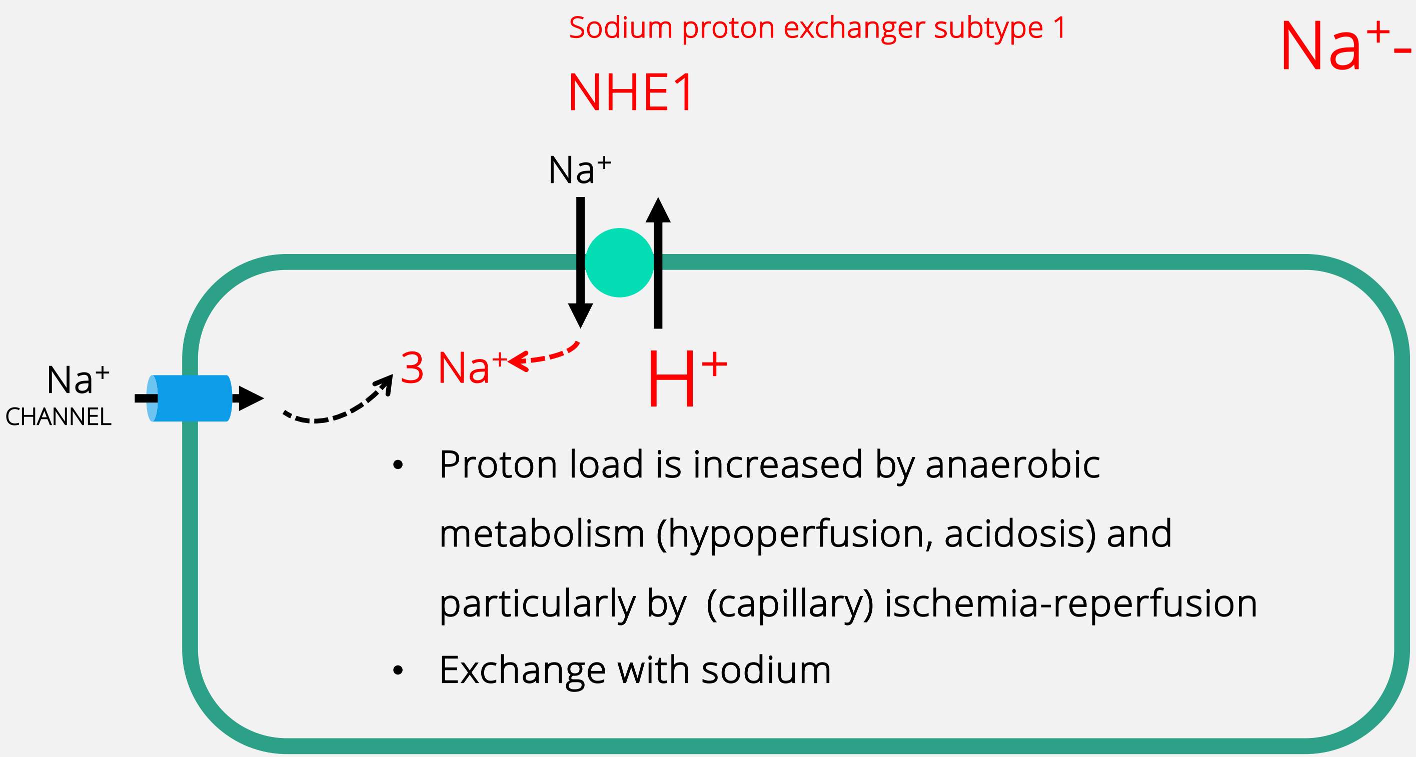


Na^{+}-INFLUX ↑

[1]Kruger et al.; 2025 Semin Thromb Hemost; Summarized in Löhn and Wirth, 2024 Medicina

PERFUSION DISTURBANCE INCREASES INTRACELLULAR PROTON CONCENTRATION IN MYOCYTES (ACIDOSIS): EXCHANGE WITH SODIUM RAISES INTRACELLULAR SODIUM

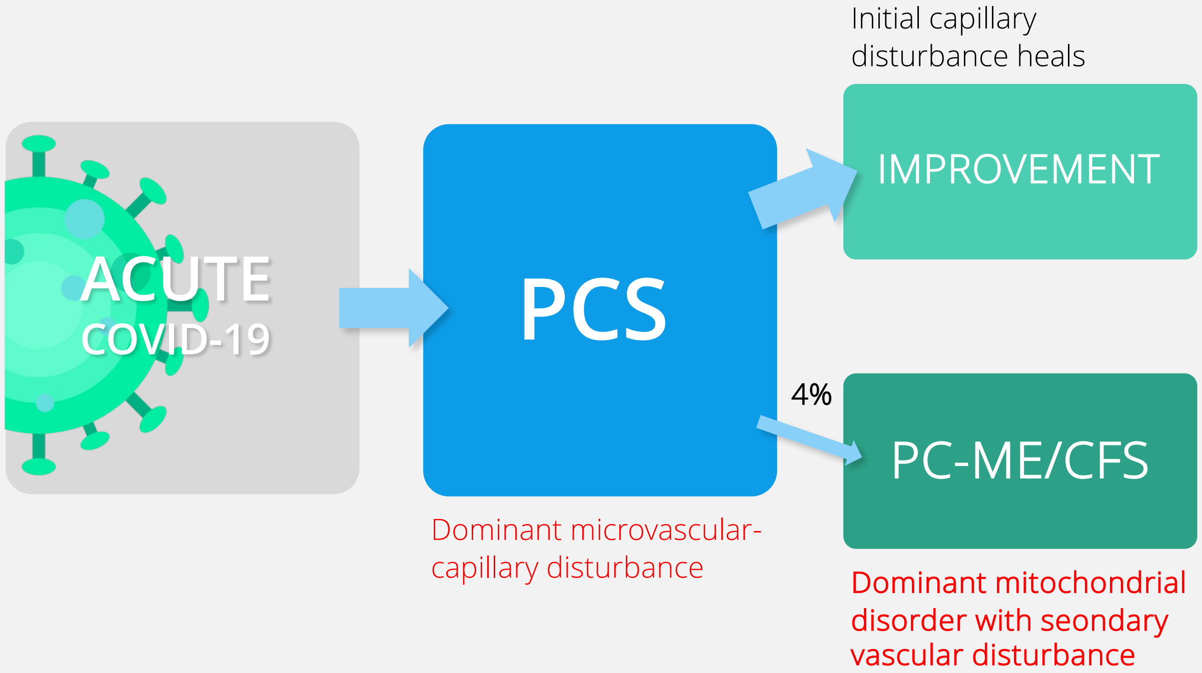
Na^+-INFLUX ↑



- Proton load is increased by anaerobic metabolism (hypoperfusion, acidosis) and particularly by (capillary) ischemia-reperfusion
- Exchange with sodium

Protons cannot freely diffuse through the cell membrane but are transporter

MOST PCS-PATIENTS SHOW IMPROVEMENT OVER TIME. WHY DOES A FRACTION OF PATIENTS DEVELOP ME/CFS? THE ROLE OF RISK FACTORS?

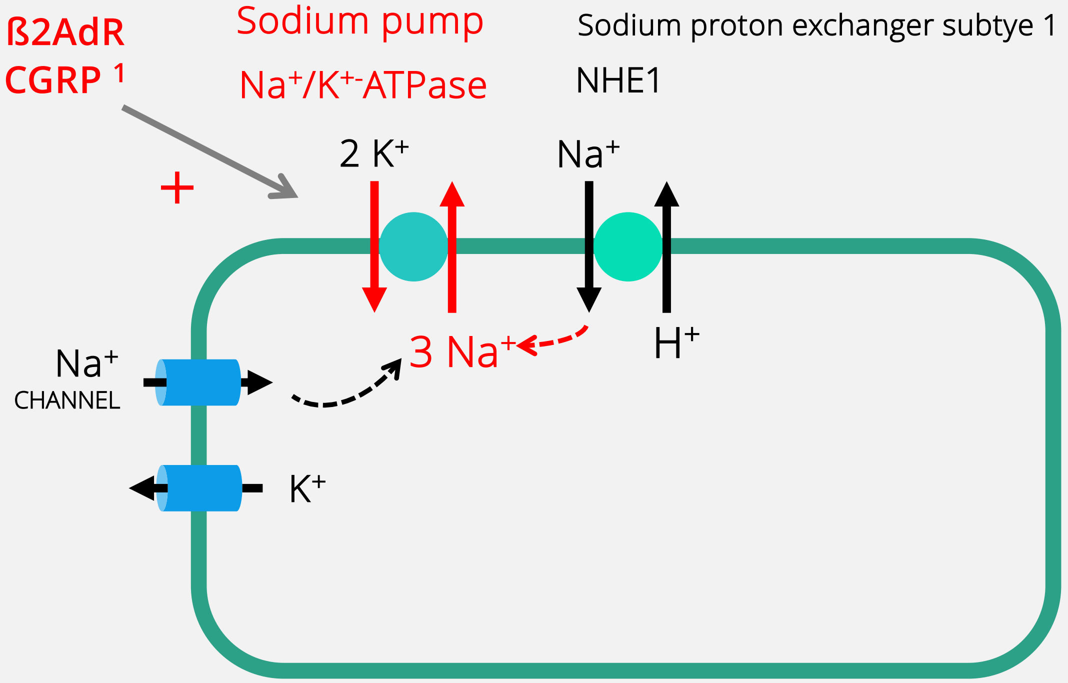


RISK FACTORS FOR ME/CFS

- Autoantibodies
- Small fiber neuropathy
- TRPM3 dysfunction
- Collagenoses
- Ehlers-Danlos syndromes
- Orthostatic dysfunction
- Variants of vascular and mitochondrial genes
- Mast cell activation
- Etc.

Mitochondrial dysfunction develops <u>due to risk factors</u> in patients with ME/CFS

RISK FACTORS FOR ME/CFS: SODIUM-EFFLUX IS ALSO DECREASED BECAUSE NA^+/K^+-ATPASE IS NOT SUFFICIENTLY STIMULATED BY ITS PHYSIOLOGICAL STIMULI



Na^+-EFFLUX ↓

Na^+/K^+-ATPase requires 10-20 fold activation during exercise[1] – high ATP consumption

The only hormonal stimuli during exercise are ß2-adrenergic receptors (ß2AdR) and Calcitionin-gene related peptide (CGRP) [1]

- ß2AdR are dysfunctional due to desensitization (high sympathetic tone) or autoantibodies
- CGRP decreased due to small nerve fiber neuropathy and TRPM3-dysfunction

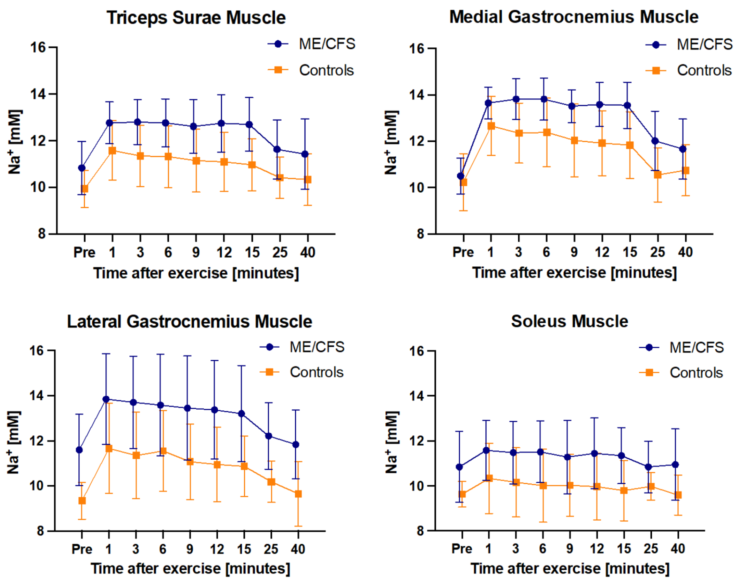
Dysfunction of both also worsens perfusion

[1] Pirkmajer and Chibalin, 2016

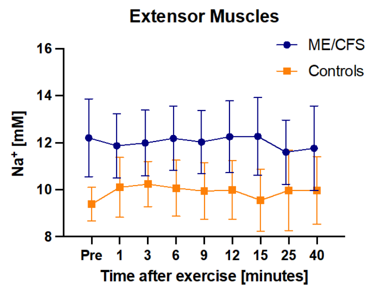
INTRACELLULAR Na^+ IS ACTUALLY ELEVATED IN SKELETAL MUSCLE IN ME/CFS

Results of a ^{23}Na Magnetic Resonance Imaging Study[1]

WORKING MUSCLE (PLANTAR FLEXION)



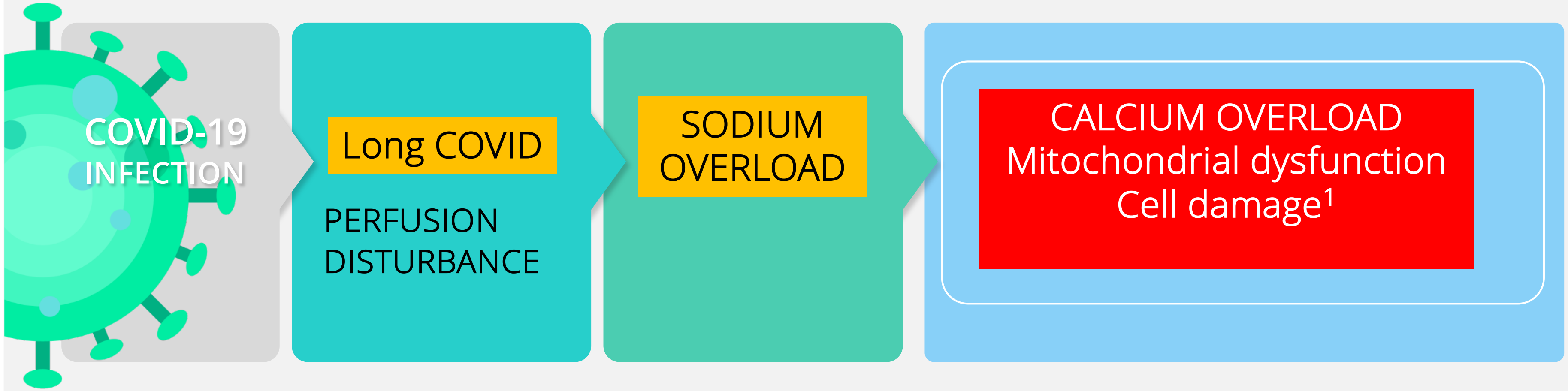
NON-WORKING LOWER LEG MUSCLE



- 6 ME/CFS patients and 6 controls.
- Approximately 20% increase in intracellular Na^+ measured before and after exercise (not during).
- Increase in intramuscular Na^+ negatively correlates with hand grip strength.
- Loss of force (hand grip strength) correlates with prognosis and symptoms in a Post COVID syndrome (PCS) study[2].

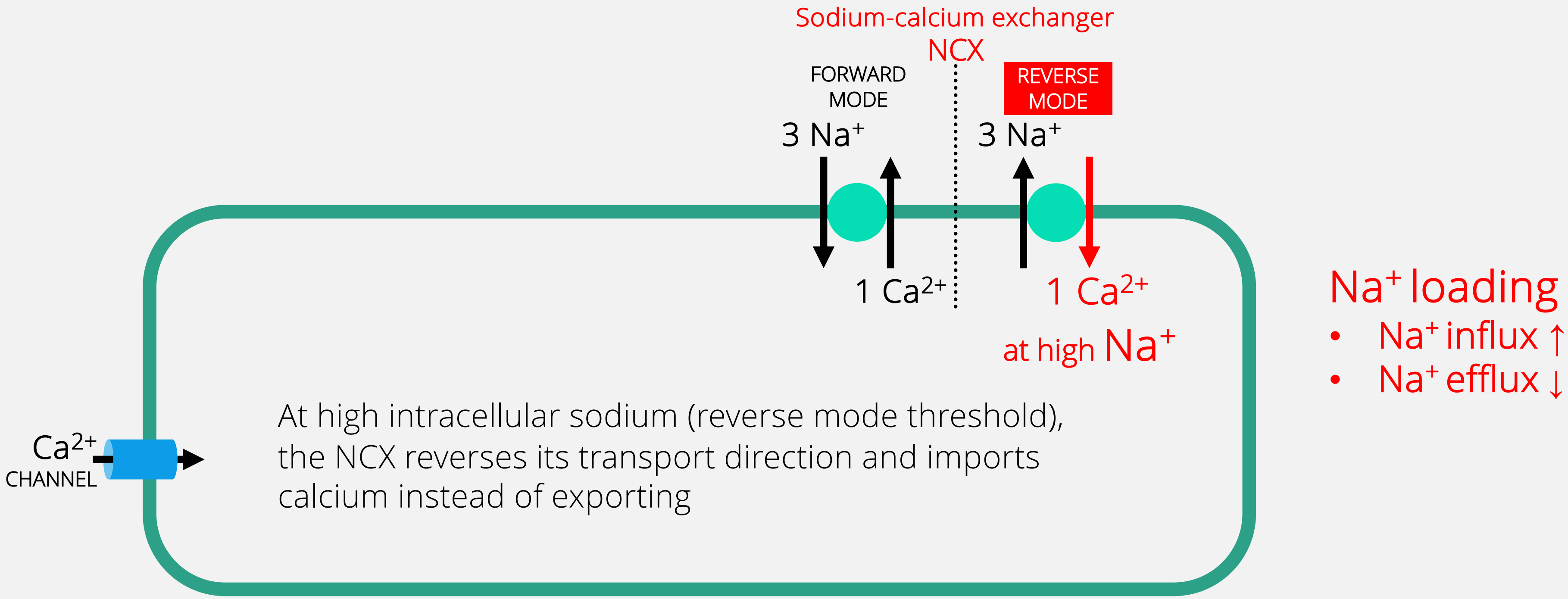
[1]Petter et al., 2022, J Transl Med. [2]Pafftrath et al. 2024. J.Clin. Med.
Triceps surae muscle: Gastrocnemius muscles (lateral and medial heads) and soleus muscle.

INTRACELLULAR SODIUM OVERLOAD CAUSES CALCIUM OVERLOAD

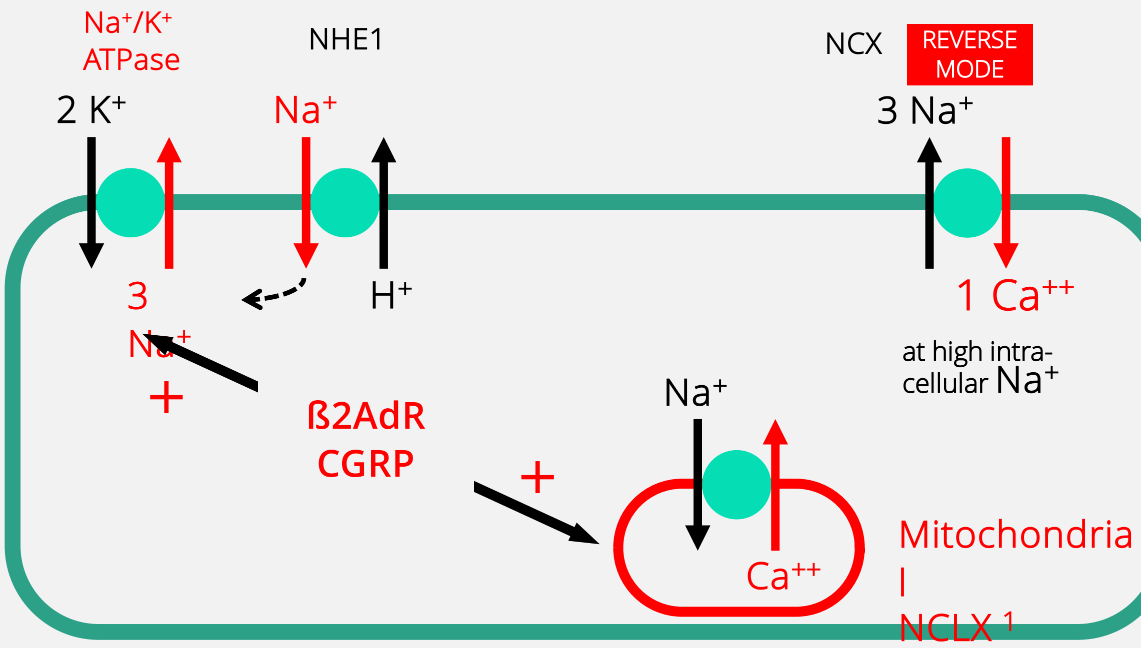


[1]Appelman et al., 2024; Nature Communications

INTRACELLULAR SODIUM OVERLOAD CAUSES CALCIUM OVERLOAD BY REVERSING THE TRANSPORT DIRECTION OF THE SODIUM-CALCIUM EXCHANGER NCX



MITOCHONDRIAL CALCIUM EXCHANGER (NCLX) IS NOT SUFFICIENTLY ACTIVATED TO PREVENT MITOCHONDRIAL CALCIUM OVERLOAD



- NCLX as the last protection against mitochondrial calcium overload fails due to dysfunctional ß2-adrenergic receptors and low CGRP
- Mitochondria become the most sensitive cellular structures for calcium overload

Mitochondrial calcium overload can even occur at moderately elevated cytosolic calcium levels

[1]Kostic et al., 2018, Cell Reports

THE THREE CONCENTRATION-DEPENDENT EFFECTS OF MITOCHONDRIAL CALCIUM

STAGE 1: CALCIUM STIMULATES MITOCHONDRIAL ATP PRODUCTION

Calcium is essential for the stimulation of mitochondrial OXPHOS stimulating enzymes of the TCA cycle.

STAGE 2: CALCIUM OVERLOAD DECREASES OXIDATIVE ATP PRODUCTION

Anaerobic glycolysis due to mitochondrial dysfunction causes sodium loading via NHE1 and to a further calcium load and mitochondrial fission.

STAGE 3: CALCIUM OVERLOAD TURNS MITOCHONDRIA INTO ATP CONSUMERS

- In the case of severe calcium overload, mitochondria can change from ATP producers to ATP consumers to maintain mitochondrial homeostasis (futile cycle).
- Severe exercise intolerance?

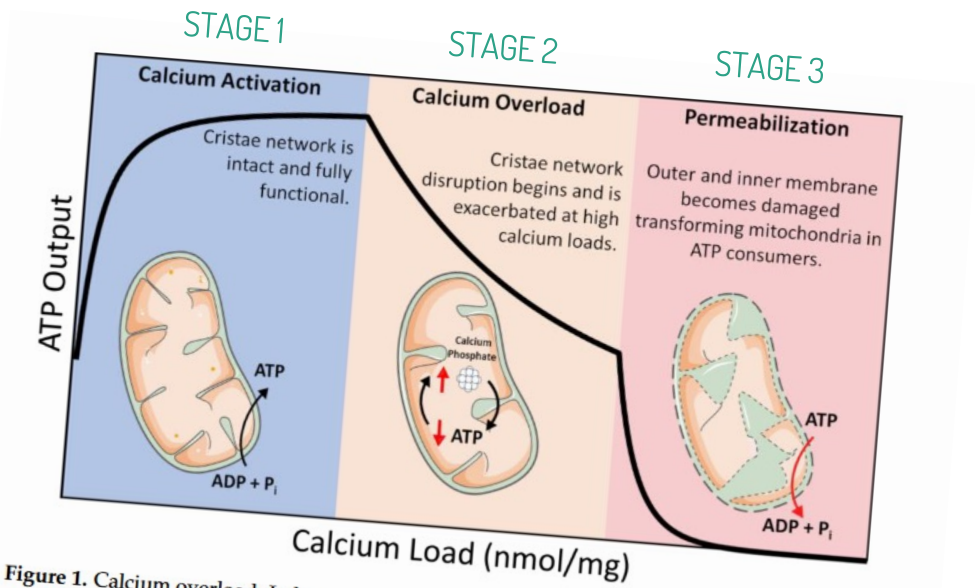
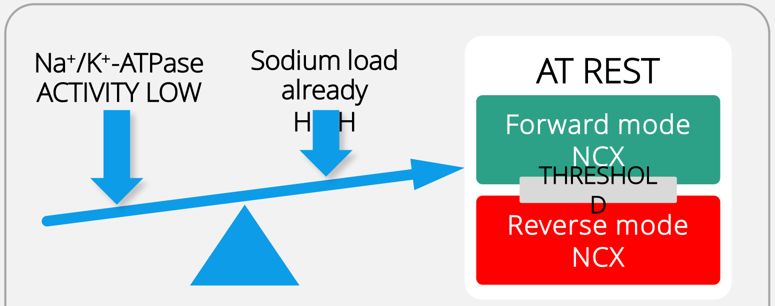


Figure 1. Calcium overload. In low amounts, calcium enhances mitochondrial function by activating several Ca^{2+}–sensitive catabolic enzymes. In moderate amounts, depressed rates of oxidative phosphorylation become observable. In extreme amounts, mitochondria become structurally compromised and consume ATP in a futile attempt to restore homeostasis.

Walkon et al., 2022, Biomolecules.

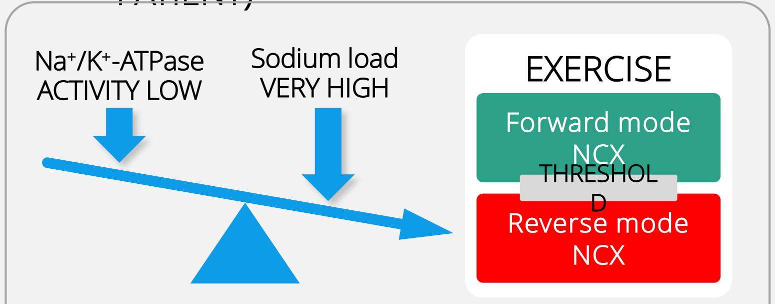
IS THE SODIUM THRESHOLD OF THE REVERSE MODE NCX THE BASE FOR THE CLINICAL PEM THRESHOLD? PATIENT IS ALWAYS CLOSE TO IT

AT REST (ME/CFS PATIENT)



- At rest, intracellular Na^+ in ME/CFS is already close to the NCX reverse mode threshold.

UNDER EXERCISE (ME/CFS PATIENT)



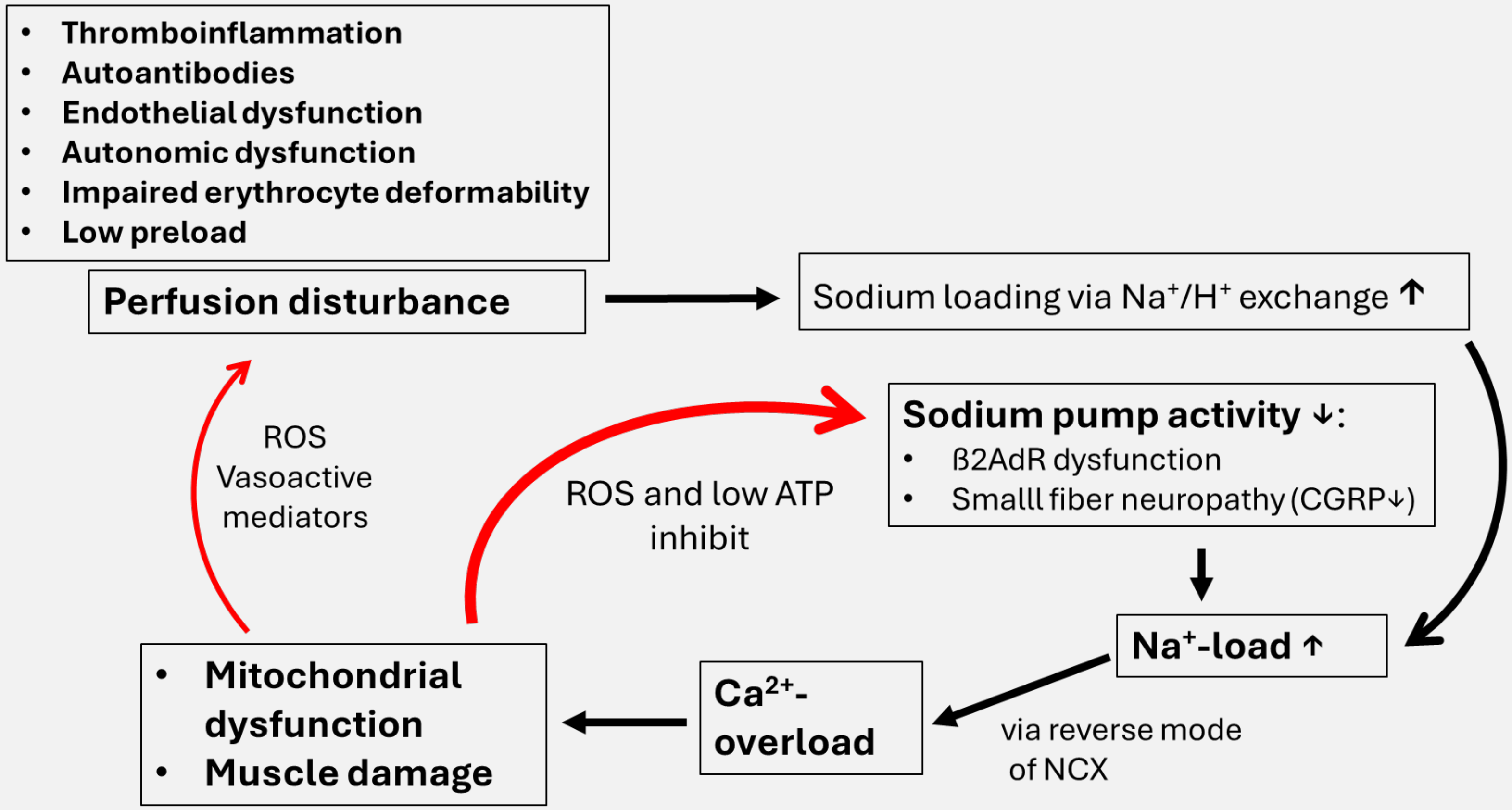
- Exercise raises Na^+ above the NCX threshold to cause Ca^{2+} overload.

⇨ Strict pacing keeps intracellular Na^+ below the reverse mode threshold to prevent calcium overload

⇨ **Drugs that keep intracellular Na^+ below the reverse mode threshold could prevent calcium overload**

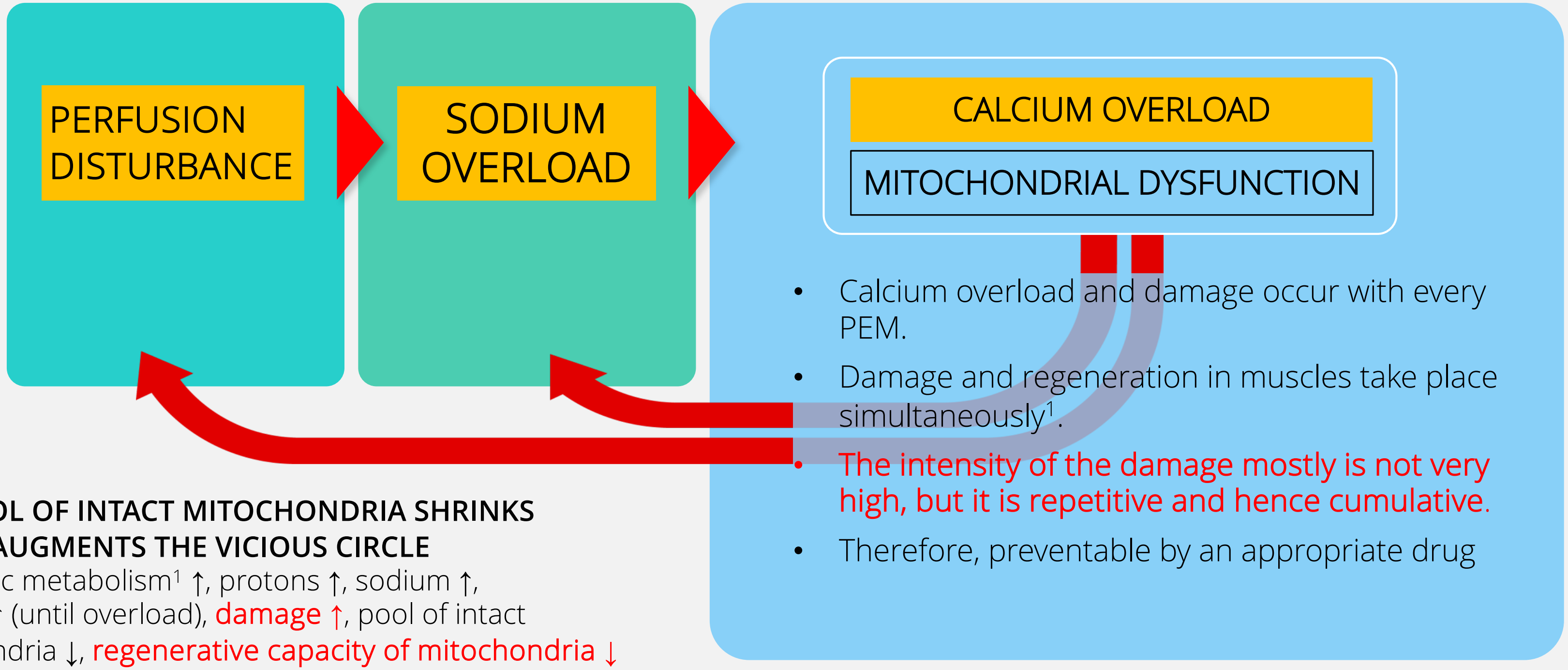
PEM: Post-exertional malaise.

MITOCHONDRIAL DYSFUNCTION LOWERS ATP AND CAUSES EXCESSIVE PRODUCTION OF REACTIVE OXYGEN SPECIES: BOTH INHIBIT NA^{+}/K^{+}-ATPASE



Scheibenbogen and Wirth, 2025; Journal of Cachexia, Sarcopenia and Muscle

CHRONICALLY THE POOL OF INTACT MITOCHONDRIA SHRINKS DUE TO REPEATED AND CUMULATIVE DAMAGE: **A VICIOUS CIRCLE THAT STABILIZES ME/CFS**



- Calcium overload and damage occur with every PEM.
- Damage and regeneration in muscles take place simultaneously[1].
- The intensity of the damage mostly is not very high, but it is repetitive and hence cumulative.
- Therefore, preventable by an appropriate drug

THE POOL OF INTACT MITOCHONDRIA SHRINKS WHICH AUGMENTS THE VICIOUS CIRCLE

Anaerobic metabolism[1] ↑, protons ↑, sodium ↑, calcium ↑ (until overload), damage ↑, pool of intact mitochondria ↓, regenerative capacity of mitochondria ↓

THERAPEUTIC STRATEGY: Inhibit damage mechanisms to enable undisturbed regeneration.

[1]Appleman et al., 2024; Nature Communications

SKELETAL MUSCLE PATHOLOGICAL FINDINGS IN PATIENTS WITH POST COVID SYNDROME WITH PEM OR ME/CFS

Arranged according to the investigational method; Scheibenbogen and Wirth, 2025, J Cachexia Sarcopenia Muscle.

CLINICAL SIGNS	SKELETAL MUSCLE FORCE TEST	EXERCISE (ENDURANCE) TEST	BIOPSIES FROM SKELETAL MUSCLE	IMAGING (MRI)
• Fatigue, early exhaustion, muscle weakness, muscle pain, fasciculations and cramps. [6-8]	• Muscular force impaired assessed via hand grip strength (HGS) and leg strength. [7] o HGS correlates with symptom severity and prognosis.	• Metabolic changes during exercise: Early appearance of anaerobic metabolism. [9] • Diminished oxygen uptake and extraction. [10]	• Skeletal muscle biopsies show signs of muscular damage and regeneration. More damage one day after exercise. [9] o Biopsies exclude obstructed capillaries, viral presence and autoimmune myositis. • Evidence for muscle mitochondrial dysfunction. [9, 13, 22, 24, 26] • Electron microscopy from skeletal muscle biopsies show changes in mitochondrial morphology and mitochondrial damage. [13, 22] o Mitochondrial damage shows a particular localization pattern. Subsarcolemmal mitochondria are affected. in contrast to interfibrillary mitochondria.	• Intracellular sodium in skeletal muscle is elevated and negatively correlated wih hand grip strength in a ^{23}Na MRI study in resting or exercising muscle depending on muscle type. [12] o Rise in intracellular sodium is a precondition for the damage by calcium. o pH changes.

ME/CFS as an acquired mitochondrial myopathy

A COMPREHENSIVE DISEASE CONCEPT SHOULD ALSO EXPLAIN THE SKELETAL MUSCLE SYMPTOMATOLOGY OF THE SEVERELY ILL ME/CFS PATIENT

- **Loss of force** and endurance (myasthenia), muscle pain, **fasciculations**, tremor and cramps
- Neurological investigations exclude motor nerve damage pointing to an intrinsic (single) muscular cause
- No atrophy of skeletal muscle[1]

Mitochondrial dysfunction (MD)

MD can explain severe fatigue and loss of endurance

- Pool of intact mitochondria may be low
- Mitochondria at high calcium can consume ATP

MD does not explain neither strong loss of force (after a rest) nor fasciculations

Disturbed Na^+/K^+-ATPase and electrophysiology

Na^+/K^+-ATPase generates excitability[2]

Insufficient Na^+/K^+-ATPase is the key assumption

Experimental inhibition of Na^+/K^+-ATPase by ouabain leads to depolarization and a total loss of muscular force[1]

<u>Dysfunction explains loss of force and fasciculations</u>

› Depolarization favors **uncontrolled excitations**

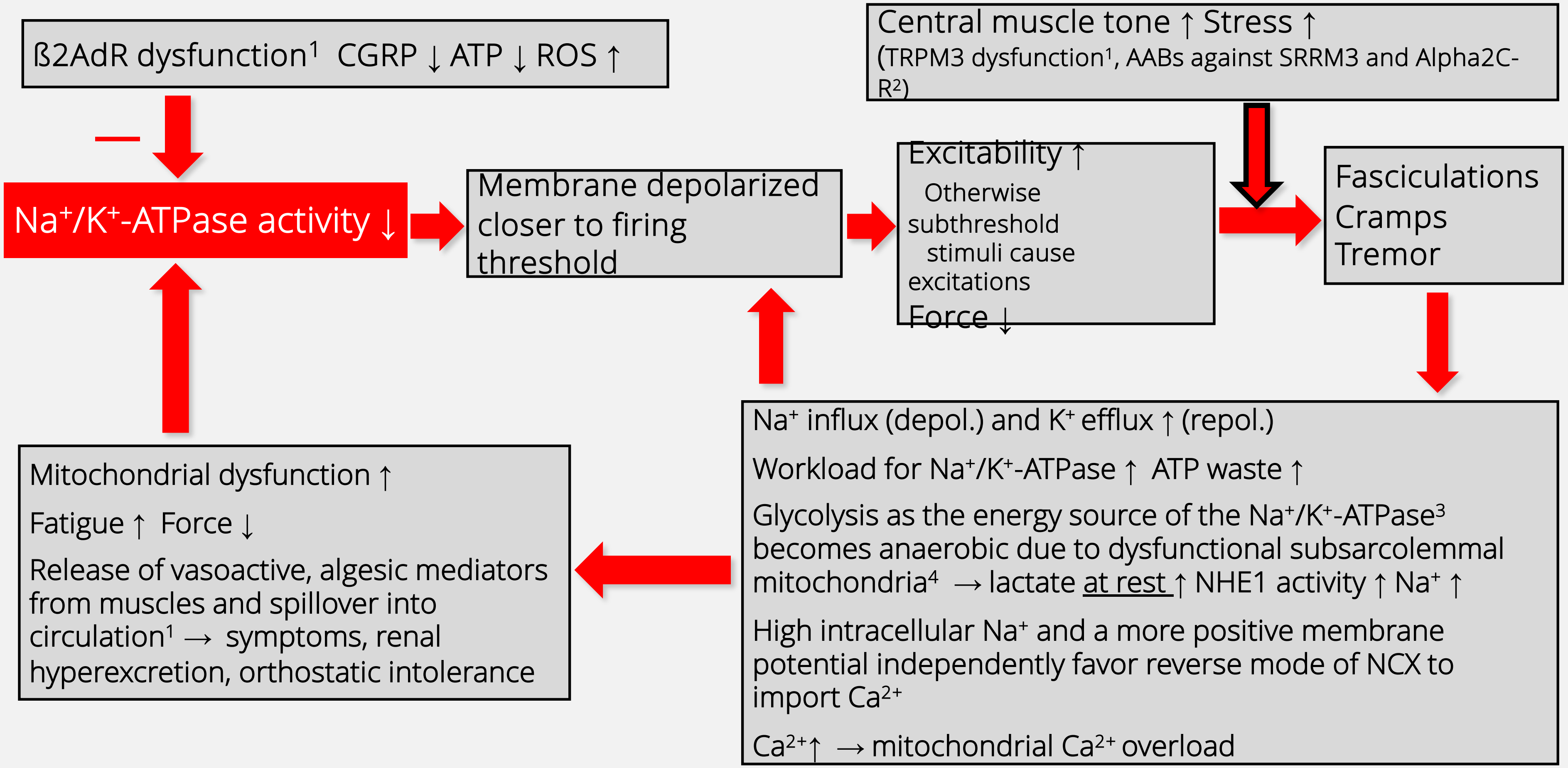
› Action potential propagation weaker (fraction of Na^+ channels in inactivated state; less muscle fibers recruited)

Key role of Na^+/K^+-ATPase explains why loss of force correlates with prognosis and symptoms

[1] Charlton et al. 2025, MedRvix preprint

[2] Pirkmajer and Chibalin, 2016

IS SKELETAL MUSCLE OF THE SEVERELY ILL IN A STATE OF DEPOLARIZATION?



[1]Wirth and Löhn, 2024 [2] Hoheisel et al., 2024 [3]Pirkmajer and Chibalin, 2016 [4]Bizjak et al., 2024

A STEP MODEL TO EXPLAIN THE SITUATION OF THE SEVERELY ILL ME/CFS PATIENT

Healthy

Long COVID

Hypoperfusion
(microvascular disturbance)

ME/CFS

+ Skeletal muscle
mitochondrial dysfunction

Disturbances induced by exercise and efforts

Severe ME/CFS

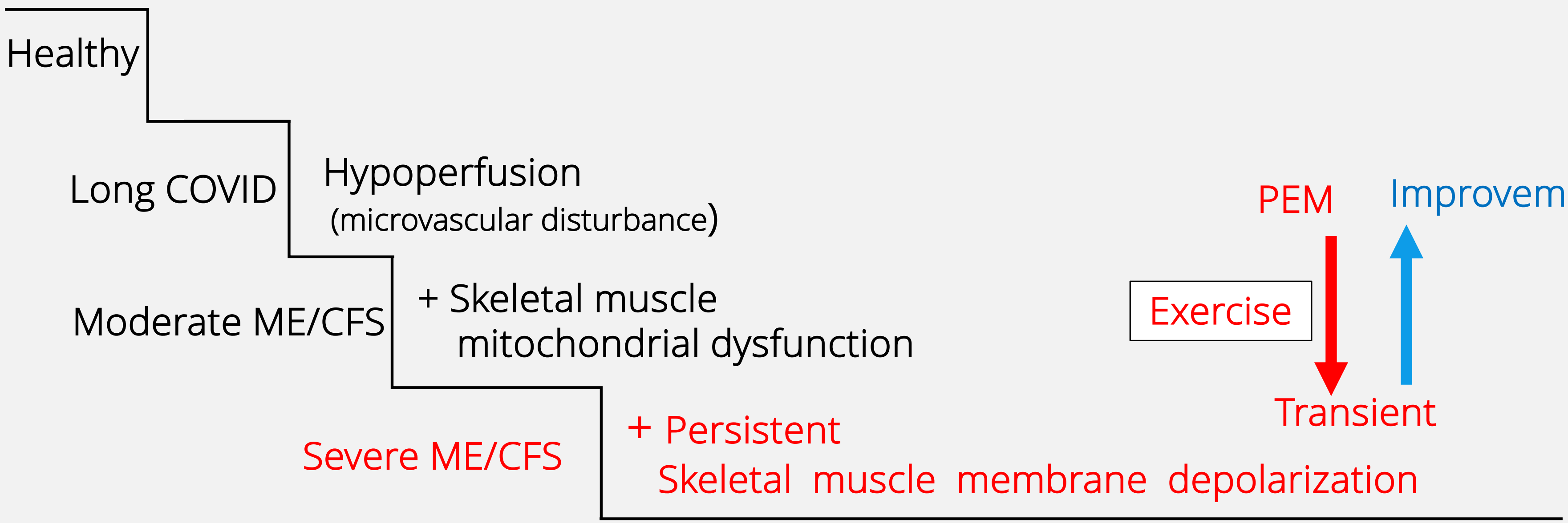
+ Persistent skeletal muscle
membrane depolarization

Disturbances operative already at rest or by minimal efforts

Insufficient Na^+/K^+ATPase activity keeps the sarcolemma in a state of depolarization

Depolarization sensitizes to central rises in muscle tone to explain why mental stress can cause muscle symptoms

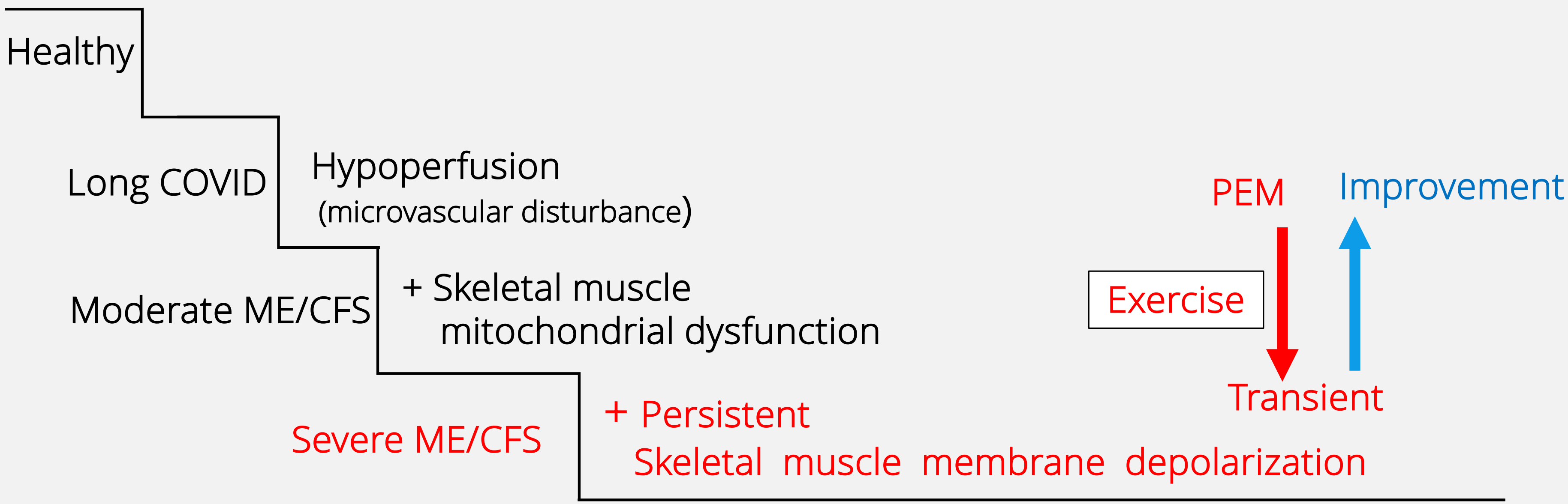
DURING PEM OF LONGER DURATION IN PATIENTS WITH MODERATE ME/CFS SKELETAL MUSCLE DEPOLARIZATION COULD ALSO BE PRESENT BUT BE TRANSIENT



M-wave alterations (EMG) were found after exercise test and correlated with loss of force[1] ("sarcolemmal fatigue"): the decreased amplitude and prolonged duration can be explained by depolarization

[1] Retornanz et al.; 2023, Clinical Biochemanics

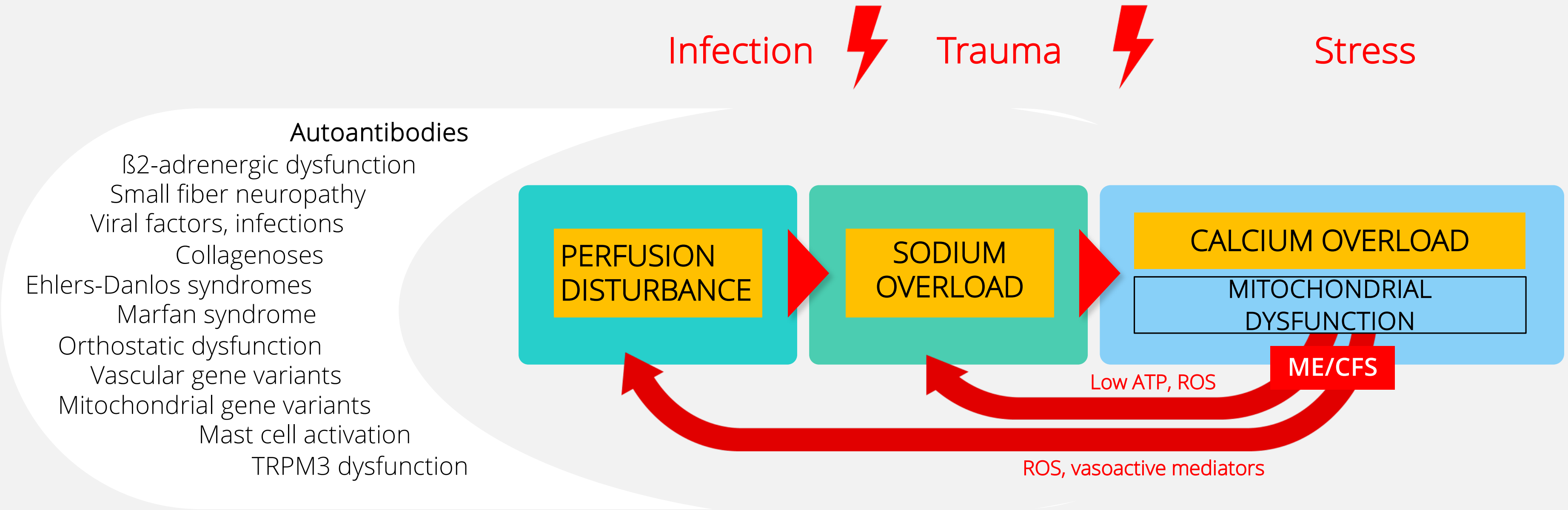
DURING PEM OF LONGER DURATION IN PATIENTS WITH MODERATE ME/CFS SKELETAL MUSCLE DEPOLARIZATION COULD ALSO BE PRESENT BUT BE TRANSIENT



M-wave alterations (EMG) were found after exercise test in a patient group with lower grip strength and more symptoms: the decreased amplitude and prolonged duration can be explained by depolarization

[1] Retornanz et al.; 2023, Clinical Biochemanics

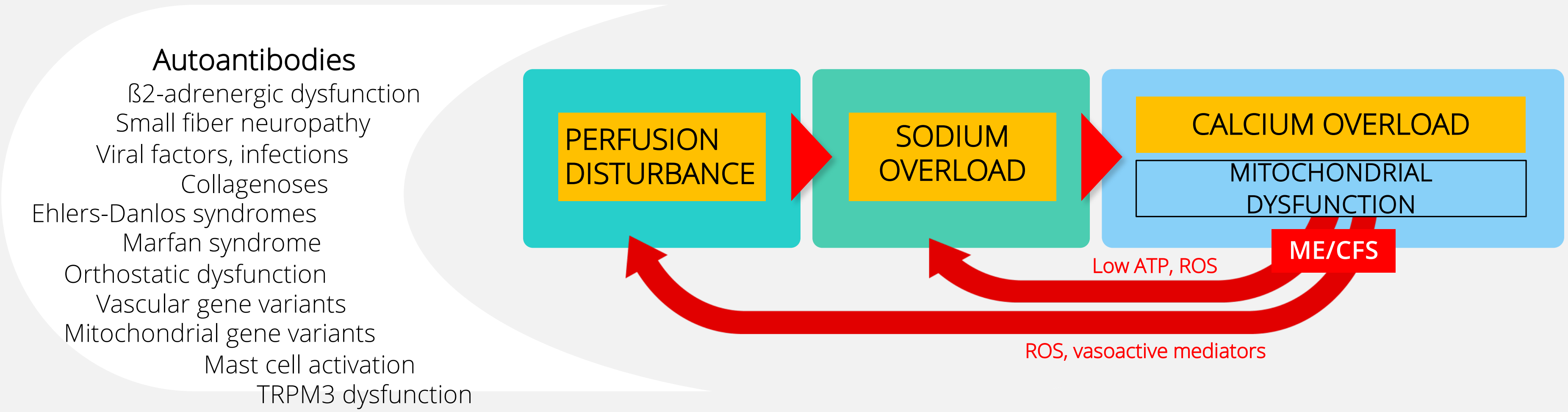
DIFFERENT TRIGGERS IN THE PRESENCE OF VARIOUS RISK FACTORS CAN CAUSE SODIUM-INDUCED CALCIUM OVERLOAD AS THE FINAL COMMON PATHWAY



Regardless of the triggering mechanism the same and stable vicious circle is operative as the final pathway = main disturbance in ME/CFS

TRIGGERS DISEAPPEAR BUT THE VICIOUS CIRCLE PERSISTS DUE TO RISK FACTORS

Particular role of autoimmunity



Disease concept seems sufficiently validated to derive a therapeutic strategy

MITODICURE 022 Science

POTENTIAL ROLE OF AUTOIMMUNITY AND AUTOANTIBODIES AS RISK FACTORS

Autoantibody target	Pathophysiological importance
Beta2 -adrenergic receptor	Sodium and calcium overload => mitochondrial dysfunction, *malperfusion* of brain and muscle.
Sodium-potassium-calcium exchanger 3 (NCKX3, SLC24A3) a variant of NCX and backup	Calcium overload => mitochondrial dysfunction.
Alpha 1 adrenergic receptor (α_1AR) => Noradrenergic activity ↑	Orthostatic intolerance and stress, preload failure, *malperfusion*, desensitization of beta 2 adrenergic receptors by orthostatic stress => mitochondrial dysfunction.
Alpha 2C adrenergic receptor (α_{2C}AR) => Noradrenergic activity ↑	Raised central noradrenergic activity - Hypervigilance, sleep disturbance, enhanced pain perception, muscle tone raised => mitochondrial dysfunction. Orthostatic stress. Sympathetic hyperactivity => desensitization of beta 2 adrenergic receptors => mitochondrial dysfunction and *malperfusion*.
Serine/arginine repetitive matrix 3 (SRRM3) => GABA activity ↓	GABA activity diminished, hypervigilance, sleep disturbance, muscle tone raised => mitochondrial dysfunction (similar to or acting in concert with TRPM3 dysfunction, which decreases the release of GABA and of GCGRP leading to calcium overload.

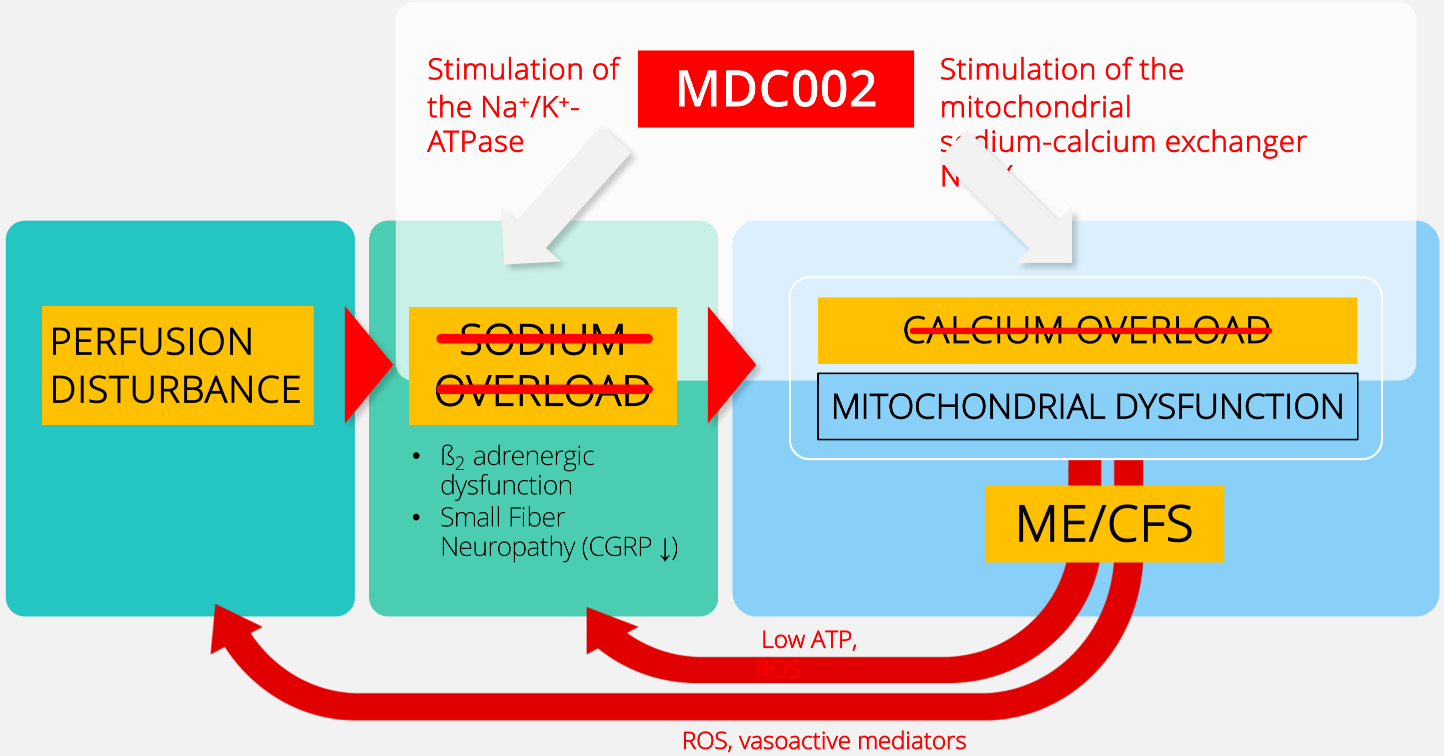
EBV*

*Autoantibodies to human proteins with high sequence homology to EBV arginine-rich (poly-R) antigen sequences

Disturbed balance between (higher) excitatory noradrenergic versus (lower) inhibitory GABA activity could explain hypervigilance, disturbed sleep, sympathetic hyperactivitiy, higher muscle tone

Hoheisel et al., 2025, Autoantibodies to Arginine-rich Sequences Mimicking Epstein-Barr Virus in Post-COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome | medRxiv

THERAPEUTIC CONCEPT: BREAKING THE VICIOUS CIRCLE THAT MAINTAINS ME/CFS AND RESTORING RESTING MEMBRANE POTENTIAL



Additional effects: MDC002 raises skeletal muscle and cerebral blood flow

CGRP: Calcitonin gene-related peptide; ROS: Reactive oxygen species