The New Paradigm in Functional Health:

A Comprehensive Approach to Optimizing Patient Outcomes

Dr. Robert Silverman

DC, DACBN, MS, CNS, CCN, CSCS, CIISN, CKTP, CES, DCBCN, HKC, FAKTR

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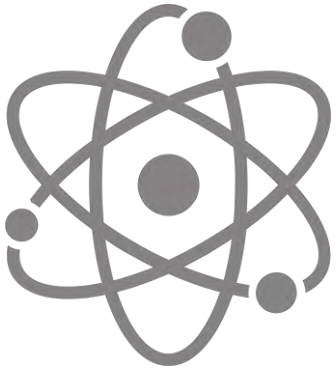
The two important days in your life are the day you were born and the day you find out why.

Mark Twain

OBJECTIVE



Science

Bridge

Application

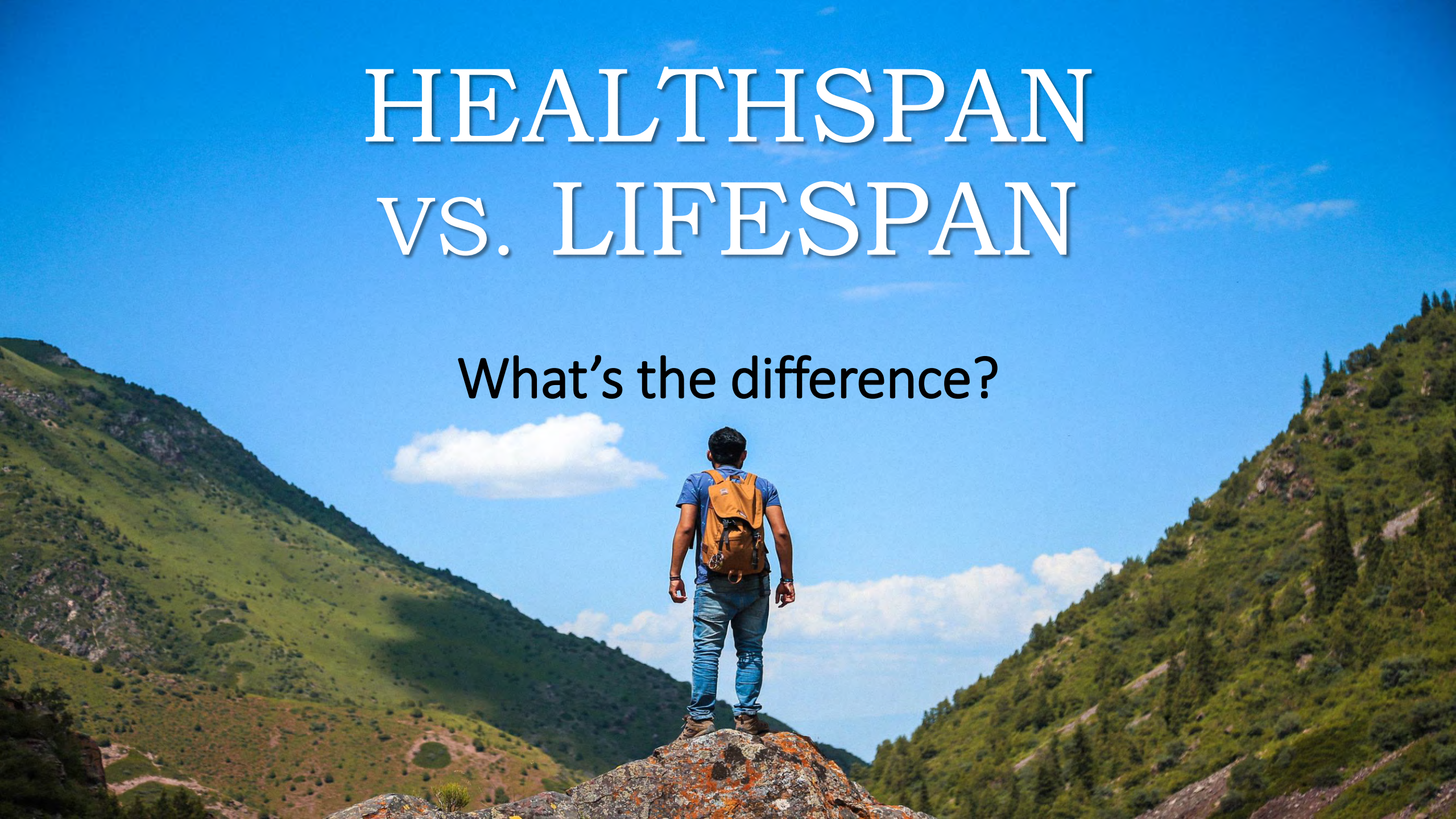
Why doesn't the old healthcare model work?

The ***primary driver*** of chronic disease is the interaction among *genes*, activities of *daily living (lifestyle)*, and the *environment*

Willett WC. Balancing lifestyle and genomics research for disease prevention. *Science*, 2002;296:695-97
Thorpe KE, Florence CS, et al. The rising prevalence of treated disease: effects on private health insurance spending. *Health Affairs*, web exclusive, June 27, 2005
Henry RP. Long-latency deficiency disease: insights from calcium and vitamin D. *Am J Clin Nutr* 2003;78:912-9

HEALTHSPAN VS. LIFESPAN

What's the difference?



“Fasting is the greatest remedy, the physician within”

Paracelsus

Table of Your Health

Financial

Family/Social

Spiritual

Individual/Wellness

INTRODUCING

GVL

By Erchonia®

The most **energetic** laser in the world.

Forecast

LLLT expected to grow from $117.1 million by the end of 2023 to $180.8 million in 2033 with a compound growth rate of 4.4%

Cold laser therapy market snapshot. *Future Market Insights*, Jan 2023

“Let there be light”

Integrating Non-Thermal Laser into Practice

"The most versatile healthcare tool of the 21st century".

Dr. Rob

Laser history

1903 Dr. Niels Ryberg Finsen – awarded Nobel Prize in Medicine and Physiology for work in treating skin tuberculosis with ultraviolet or blue light and smallpox with red light

https://www.britannica.com/biography/Niels-Ryberg-Finsen

5 Reasons…

1) Effective – “The speed of light”
2) Research-driven; empirically studied. FDA-cleared
3) Practice building
4) Joint health
5) Brain health

Laser Focus

- Laser: Light Amplification by Stimulated Emission of Radiation
- A focused beam of light that emits photon energy
- All photons travelling same direction at same wavelength = coherent light

What is LLLT

- AKA photobiomodulation
- Low intensity light therapy
- Effect: photochemical, not thermal
- Light triggers biochemical changes within cells
- Can be compared to photosynthesis in plants
- Photons absorbed by cellular photoreceptors and triggers chemical changes

HB Cother, RT Chow, MR Hamblin, J Carroll. *MOJ Orthop Rheumatol.* 2015;2(5):00068. Published online 2015 Jun 9

Laser directed to affected site

Photon Enters Tissue
Alters Cell Permeability

↓

Cellular Photochemical Reaction
Absorbed into Mitochondria → ATP+

↓

Resulting Effects

Rapid Cell Growth → Increased Metabolic Activity

Increased Angiogenesis → Vascular Activity

Suppression of COX-2 Pathway → Decreased Inflammation

Laser Therapy

5 components integral to beneficial outcome

LASER

1) Active ingredient

Specific wavelength (color) - component responsible for influencing biochemical cascades

2) Dosage

Intensity (power of light) determines response. Too little limits response. Too much produces adverse effect

3) Delivery Mechanism

Manner light is delivered determines proper tissue response and depth of penetration. Coherent, focused light insures deep tissue stimulation and absorption

4) Movement (advanced)

Turns muscle on and off.
Move handheld.
Built-in – FX635/405

5) Pulsing

Deliver shorter pulses of energy

Could Non-Thermal Laser (NTL) be the answer and why?

- Non-invasive
- No downtime
- No pain
- Short treatment time
- Pain-relieving properties
- Decreases swelling
- Improves blood flow
- Enhances energy production
- Optimizes mitochondrial function
- Anti-inflammatory
- Immune boosting properties
- Promotes stem cell production
- Decreases stress hormones
- Neuroprotective
- Down-regulates stress responses in brain
- Accelerates wound-healing
- Upregulates collagen production
- Fat loss
- Cellulite reduction
- Skin conditions

Mitochondria Health is Linked to Aging

The Mitochondrial Basis of Aging

Nuo Sun,[1] Richard J. Youle,[2] and Toren Finkel[1]

Review

Cell Death and Inflammation: The Role of Mitochondria in Health and Disease

Anna Picca [1,2], Riccardo Calvani [1,2,*], Hélio José Coelho-Junior [3] and Emanuele Marzetti [1,3]

Mitochondria and Inflammation: Cell Death Heats Up

Esmee Vringer[1,2] and Stephen W. G. Tait[1,2]

[1] Cancer Research UK, Beatson Institute, Glasgow, United Kingdom, [2] Institute of Cancer Sciences, University of Glasgow, Glasgow, United Kingdom

Review article

Inflammation and mitochondrial dysfunction: A vicious circle in neurodegenerative disorders?

Jack van Horssen *, Pauline van Schaik, Maarten Witte

Dept. of Molecular Cell Biology and Immunology, VU University Medical Center, Amsterdam, The Netherlands

Mitochondrial DNA in inflammation and immunity

Joel S Riley[1,2,*] & Stephen WG Tait[1,2,**]

The Aging Mitochondria

Pierre Theurey [1], Paola Pizzo [2] [3]

Can This Technology Vastly Decrease Opioid Use

FDA Grants 510(k) Market Clearance for Whole Body Postoperative Pain to World Leader in Low Level Laser Technology

Efficacy of 635nm Red Low-Level Laser on Nociceptive Musculoskeletal Pain Compared to NSAIDS, Opioids, and Other Light Sources

Erchonia's Published Peer-Reviewed Results Reveal Promising Treatment for Musculoskeletal Pain

A sample size of over 400 subjects, a p-value of .00001, multiple blinded & controlled studies shows 635nm red lasers are an effective treatment

Two Randomized, Double Blind, Placebo-Controlled Trials Evaluating the Efficacy of Red 635nm Low Level Laser for the Treatment of Low Back Pain

Low-Level Laser Therapy for Treating Low Back Pain: 12-Month Follow-Up

Trevor S. Berry[1], Paul J Quarneri[2], Gregory Roche[3] and Travis M Sammons[4]*

[1]South Mountain Chiropractic Center, Chandler, AZ, USA
[2]Quarneri Chiropractic Inc., San Mateo CA, USA
[3]Bloomfield Laser & Cosmetic, Bloomfield Hills, MI, USA
[4]Erchonia Corporation, Melbourne, FL, USA

Chronic Pain & Non-Thermal Laser

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How Trauma & Chronic Pain Change The Autonomic Nervous System (ANS)

- The ANS essentially impacts all organs and tissues throughout the body, which is why we see virtually all these outcomes with trauma and aging.
- During a traumatic event and/or chronic stress response, massive amounts of catecholamines are released, triggering inflammatory responses and immune dysfunction.
- This response may trigger multiple responses, usually involving increased sympathetic activity and eventual immune system depression.
- We also see a sympathetic wind-up of central circuits in the brain that promotes more pain, inflammation, and autonomic dysfunction.
    - Sympathetic vs. Parasympathetic…

Autonomic Dysfunction after Mild Traumatic Brain Injury

Dmitry Esterov[1,*] and Brian D. Greenwald[2]

Can this Technology Have Beneficial Results in Treating Neurodegeneration

Low-level Laser Therapy for Beta-Amyloid Toxicity in Rat Hippocampus

Assessing The Autonomic Effect Of Vagal Nerve Stimulation With Low Level Lasers By Heart Rate Variability

C Machado, Y Machado, M Chinchilla, Y Machado, H Foyaca-Sibat

Protection against neurodegeneration with low-dose methylene blue and near-infrared light

May 2015 · Frontiers in Cellular Neuroscience 9(36)

DOI:10.3389/fncel.2015.00179

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Vagal Nerve Stimulation With Low Level Lasers Of Two Different Frequencies, Assessed By QEEG

C Machado, Y Machado, M Chinchilla, Y Machado, H Foyaca-Sibat

Low-Level Laser Therapy Applied Transcranially to Mice following Traumatic Brain Injury Significantly Reduces Long-Term Neurological Deficits

AMIR ORON,[1] URI ORON,[2] JACKSON STREETER,[2] LUIS DE TABOADA,[2] ALEXANDER ALEXANDROVICH,[3] VICTORIA TREMBOVLER,[3] and ESTHER SHOHAMI[3]

Treating cognitive impairment with transcranial low level laser therapy

Jack C. de la Torre

Can this Technology be seen as Promising for Neurodevelopmental and Mental Health Disorders

Article

Transcranial Photobiomodulation for the Treatment of Children with Autism Spectrum Disorder (ASD): A Retrospective Study

Stefano Pallanti [1,2,*], Michele Di Ponzio [1], Eleonora Grassi [1], Gloria Vannini [1] and Gilla Cauli [3]

Effects of Low-Level Laser Therapy in Autism Spectrum Disorder

Gerry Leisman, Calixto Machado, Yanin Machado, and Mauricio Chinchilla-Acosta

Follow-Up Assessment Of Autistic Children 6 Months After Finishing Low Lever Laser Therapy

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Low-Level Laser Irradiation Improves Depression-Like Behaviors in Mice

Zhiqiang Xu [1 2], Xiaobo Guo [2], Yong Yang [3], Donovan Tucker [4], Yujiao Lu [4], Ning Xin [1], Gaocai Zhang [1 2], Lingli Yang [1], Jizhen Li [5], Xiangdong Du [3], Quanguang Zhang [6], Xingshun Xu [7 8]

Non-Thermal Low-Level Laser & Brain Health

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Non-Thermal Laser is Promising for Neurodevelopmental and Mental Health Disorders

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Low-level laser prolong longevity of degenerative knee joints

- 70 elderly patients
- Bilateral tricompartmental knee arthritis
- One knee per patient received laser plus therapy
- Other knee received PT and sham light
- Laser group 1 in 70 needed joint replacement
- Sham light 15 in 70 needed joint replacement

Clin Interv Aging, Aug 5, 2015. 10:1255-8

LLLT for thyroid

- All patients who received LLLT – able to reduce levothyroxine dose, while 47% were able to discontinue levothyroxine and have normal thyroid function during 9-month follow up
- LLLT can:
  - Increase Transforming Growth Factor B (TGF-B)
  - Increase circulation in the gland

1. Hofling DB, et al. **Effects of Low-Level Laser Therapy on the serum TGF-B1 Concentrations in individuals with autoimmune thyroiditis**. *Photomedicine and laser Surgery*, 2014;32:8

2. Hofling DB et al. **Low-level laser in the treatment of patients with hypothyroidism induced by chronic autoimmune thyroiditis: a randomized, placebo-controlled clinical trial**. *Lasers Med Sci,* 2013;28:743–753

3. Holding DB et al. **Low-level laser therapy in chronic autoimmune thyroiditis: A Pilot Study**. *Lasers in Surgery and Medicine,* 2010;42:589-596

4. Hofling DB. **Assessment of the Effects of Low-Level Laser Therapy on the Thyroid Vascularization of Patients with Autoimmune Hypothyroidism by Color Doppler Ultrasound**. *ISRN Endocrinology*, 2012

PBM in human muscle tissue: an advantage in sports performance

Results:

- Laser therapy applied before or after sports activity had profound impact on performance
- Results were as if athletes had taken performance enhancing drugs and they questioned if they should even be allowed in international competition as they seemed to provide an “unfair advantage”

Ferraresi C, Huang YY, Hamblin MR. **Photobiomodulation in human muscle tissue: an advantage in sports performance?.** *J Biophotonics*. 2016;9(11-12):1273-1299

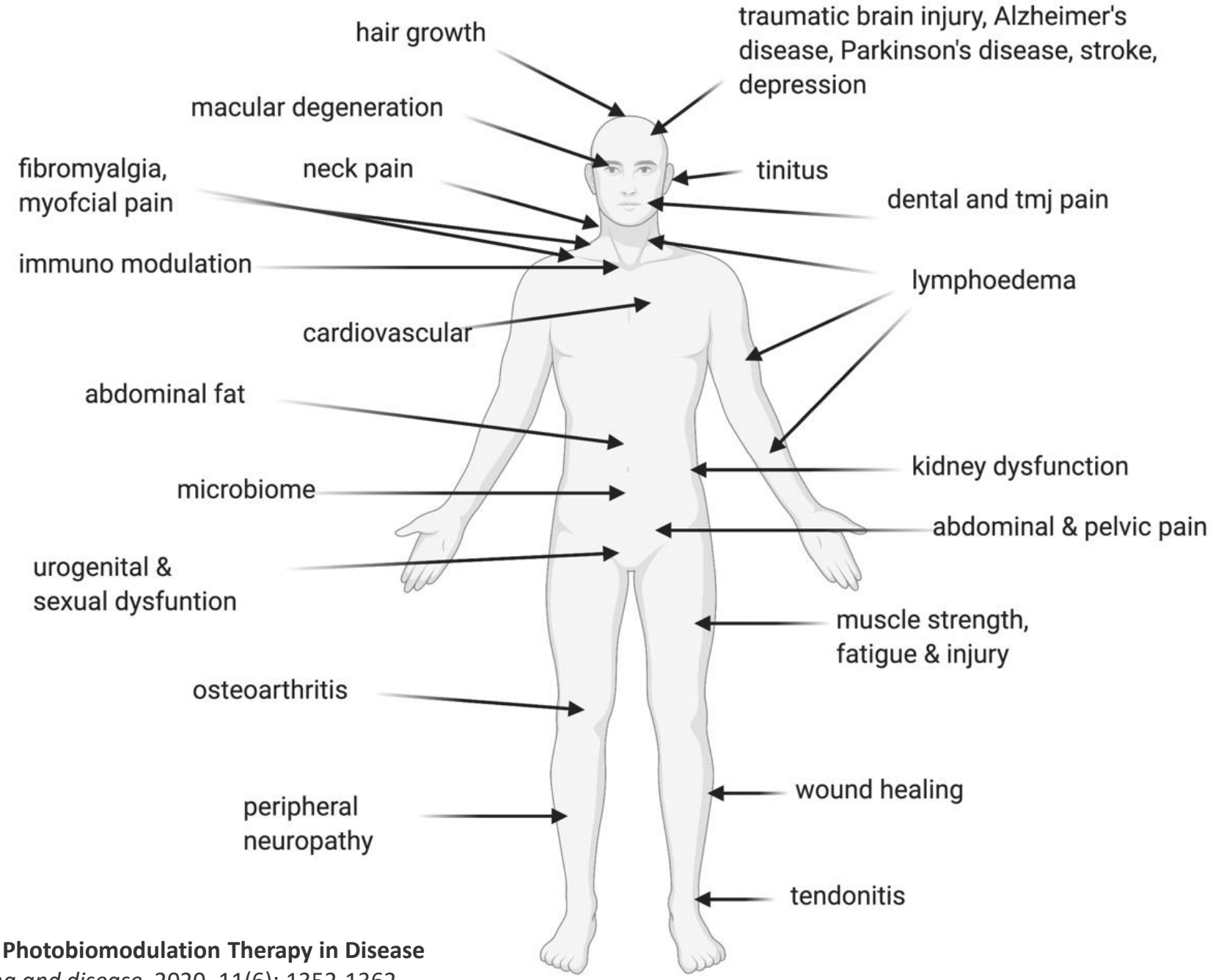
Effect of LLLT on serum vit. D and mg. levels in patients with diabetic peripheral neuropathy

Result:

Significant increase in vitamin D and magnesium levels after LLLT

Anju M, Chacko L, Chettupalli Y, et al. **Effect of Low Level Laser Therapy on serum vitamin D and magnesium levels in patients with diabetic peripheral neuropathy - A pilot study**. *Diabetes Metab Syndr*. 2019 Mar-Apr;13(2):1087-1091

Conditions that have been shown to be successfully treated using photobiomodulation therapy



Ann L, Brian B, Wayne M, Hosen K. **A Potential Role for Photobiomodulation Therapy in Disease Treatment and Prevention in the Era of COVID-19**. *Aging and disease*. 2020, 11(6): 1352-1362

Efficacy of red LLLT for postoperative pain management

Conclusion:

- Red LLLT – FDA-cleared for treatment of postoperative pain relief, based on safety and effectiveness across various surgical types
- In addition to postoperative pain reduction, clinical research demonstrated – red LLLT may promote healing and reduce consumption of analgesic drugs

Sammons T, Shanks S. **Efficacy of red low-level laser for postoperative pain management: A review of literature.** *J Perioper Pract*. 2022 Nov 17:17504589221124757

Laser pain relief

Conclusion:

- 632.5 nm laser may inhibit emergence of chemotactic factors in early stages of inflammation
- Also, inhibiting COX-2/COX-1
- Cox inhibitors and laser both inhibit expression of COX enzyme and PGE_2 release
- **Red light application decreased ROS level and $cPLA_2$**

WB Lim, SG Lee, I Kim, et al. **The anti-inflammatory mechanism of 635 nm light-emitting-diode irradiation compared with existing COX inhibitors.** Lasers Surg Med, Aug 2007;39(7):614-21

Laser wound healing

- Low doses of red light – affect the expression of 111 genes related to microcirculation, antioxidation and DNA repair, leading to acceleration of the proliferation of fibroblast and endothelial cells in wound healing

Sammons T, Shanks S. **Efficacy of red low-level laser for postoperative pain management: A review of literature.** *J Perioper Pract*. 2022 Nov 17:17504589221124757

Laser healing

- Red 635 nm laser exhibited ability to reduce oxidative stress that occurs during the inflammation phase in the muscle repair process by down regulating the expression of TGF-β1
- Fibrotic area of 635 nm LLLT group – significantly smaller that of control group 14, 21, and 28 days after injury

L Luo, ZW Sun, L Zhand, et al. **Effects of low-level laser therapy on ROS homeostasis and expression of IGF-1 and TGF-β1 in skeletal muscle during the repair process**. *Lasers Med Sci* (2013) 28:725–734

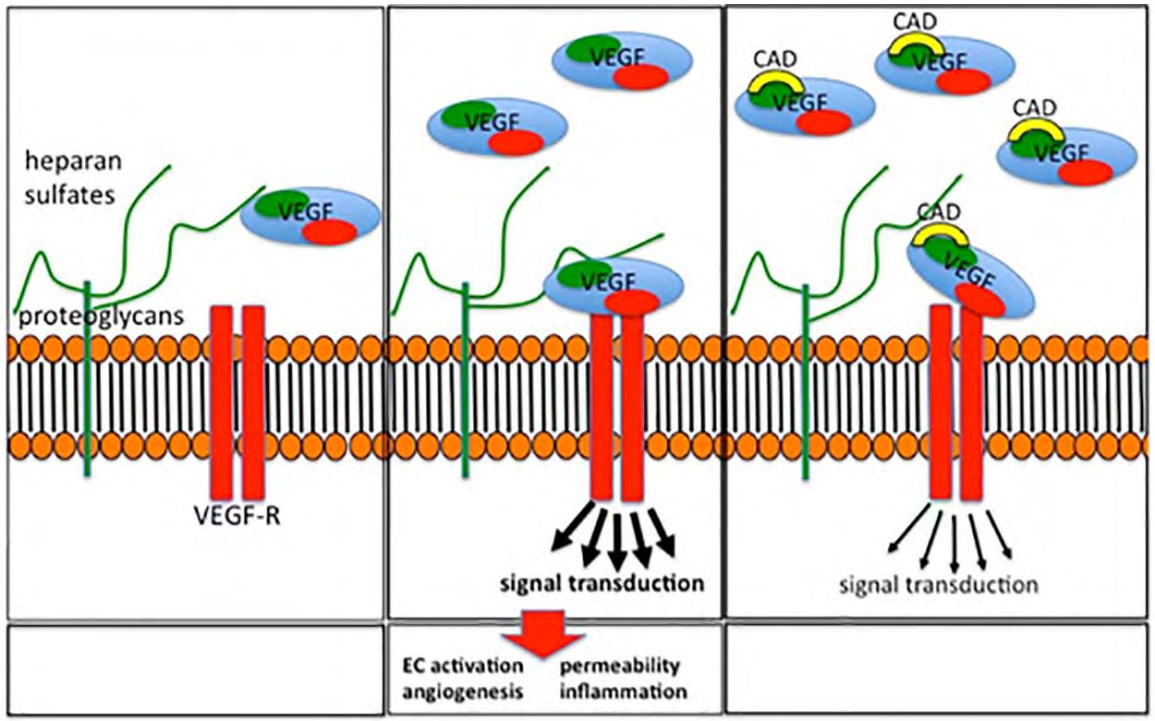
Laser/healing

- Red laser – significantly raised IL-10 doses
- As IL-10 increases:
  - Inhibits release of IL-6, IL-8, IL-1β, TNF
  - Regulates switch of M1 to M2 phenotype in injured muscle

Sammons T, Shanks S. **Efficacy of red low-level laser for postoperative pain management: A review of literature.** *J Perioper Pract*. 2022 Nov 17:17504589221124757

Laser/blood vessels

- Red laser radiation at wavelength of 630 nm can cause statistically significant increase of about 30% in VEGF gene expression in vascular endothelial cells compared with that of control cells



doi: https://doi.org/10.1371/journal.pone.0218494.g010

Medical Research Journal, 9 Nov 2014;2(2):61–65

LLLT regulates the remodeling of ECM

The remodeling of ECM	Factors associated with the process
Protein synthesis	TGF-β, MMP-2, MMP-3, and MMP-14
Protein degradation	TGF-β, MMP-2, MMP-3, MMP-9, MMP-13, and MMP-14

ECM, extracellular matrix; TGF-β, transforming growth factor-β; MMP-2, matrix metalloprotease 2; MMP-3, matrix metalloprotease 3; MMP-9, matrix metalloprotease 9; MMP-13, matrix metalloprotease 13; and MMP-14, matrix metalloprotease 14.

The Functions and Mechanisms of Low-Level Laser Therapy in Tendon Repair (Review). *Front Physiol*, 15 Feb 2022; 13

LLLT downregulates diverse inflammatory cytokines

	Different cytokines associated with an inflammatory response
Damage stage	• M1 macrophages, neutrophils, TNF-α, IL-6, IL-1β, PGE2, Cox-2, and NF-kB pathway
Repair phase	• M2 macrophages, Cox-7, and IL-10

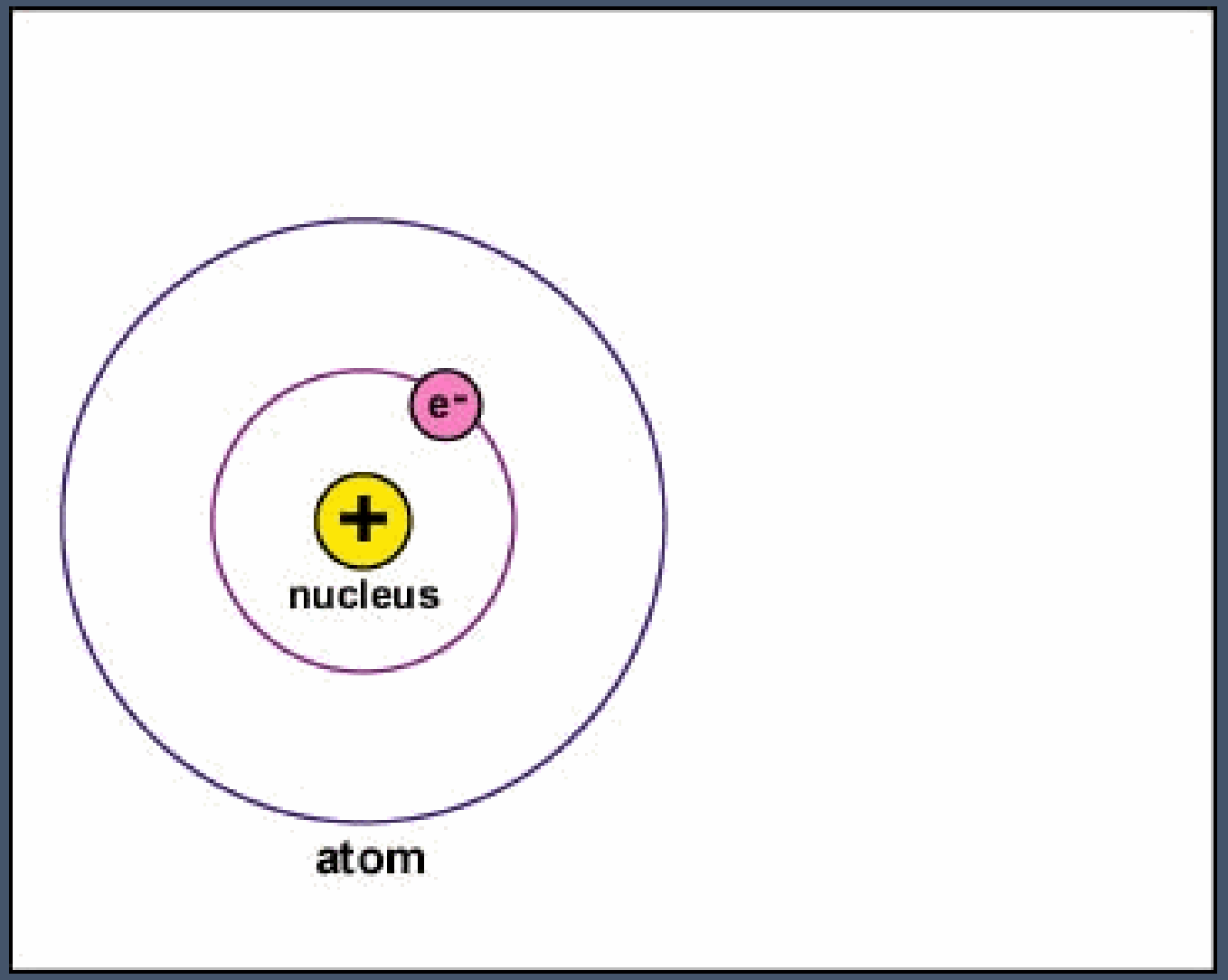
LLLT, low-level laser therapy; TNF-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-1β, interleukin-1β; PGE2, prostaglandin E2; Cox-2, cyclooxygenase 2; NF-kB pathway, nuclear factor kappa-B; Cox-7, cyclooxygenase 7; and IL-10, interleukin-10.

The Functions and Mechanisms of Low-Level Laser Therapy in Tendon Repair (Review). *Front Physiol*, 15 Feb 2022; 13

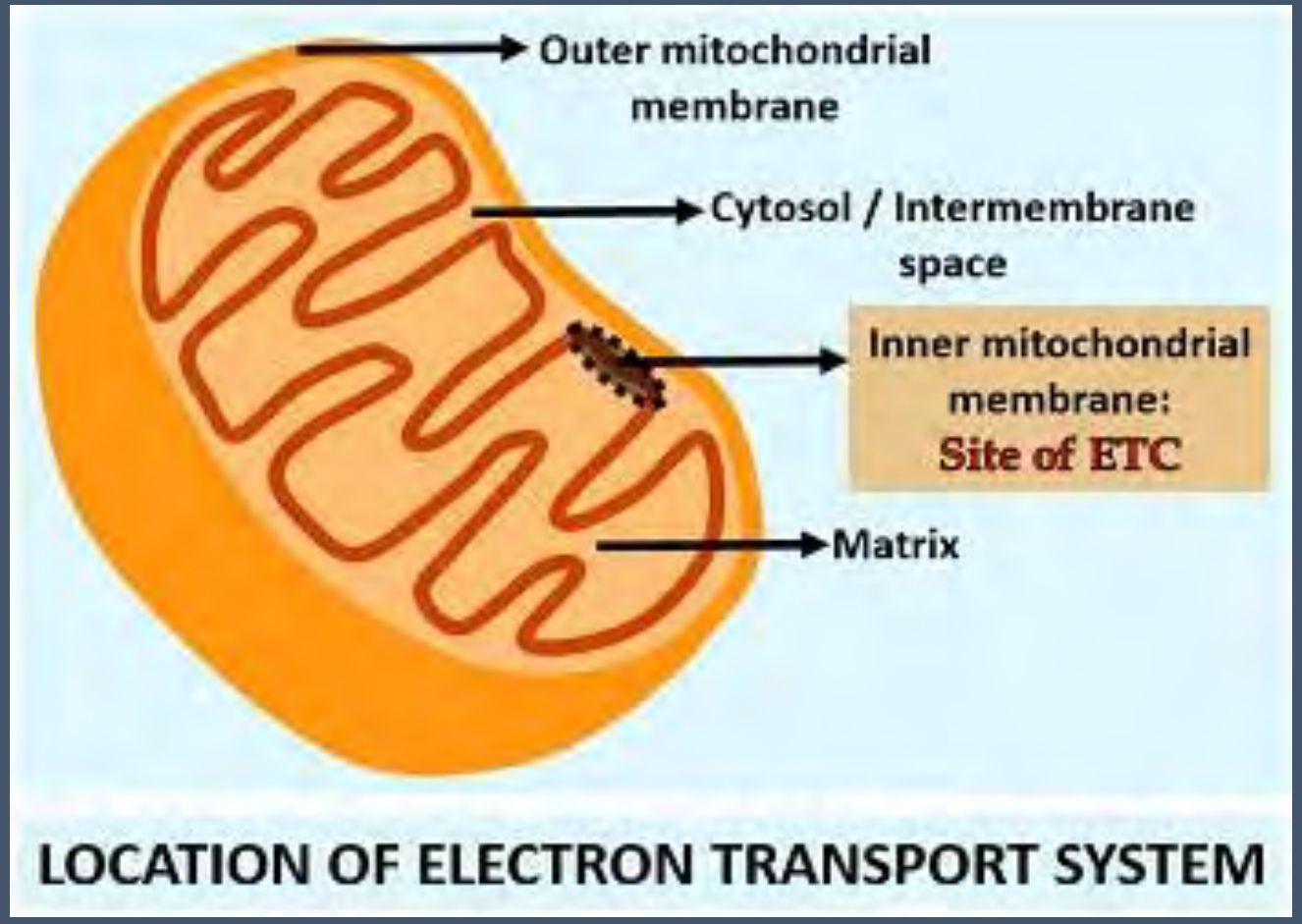
Electron Transport Chain

Electron transport chain uses high-energy electrons **to convert ADP into ATP**. High-energy electrons from NADH and $FADH_2$ are passed along electron transport chain from one carrier protein to the next.

When photons of visible light energy strike certain atoms, that energy may push an electron from that atom to a higher energy level where it can be picked up by an electron acceptor in an electron transport chain.



What produces the ATP in the mitochondria?



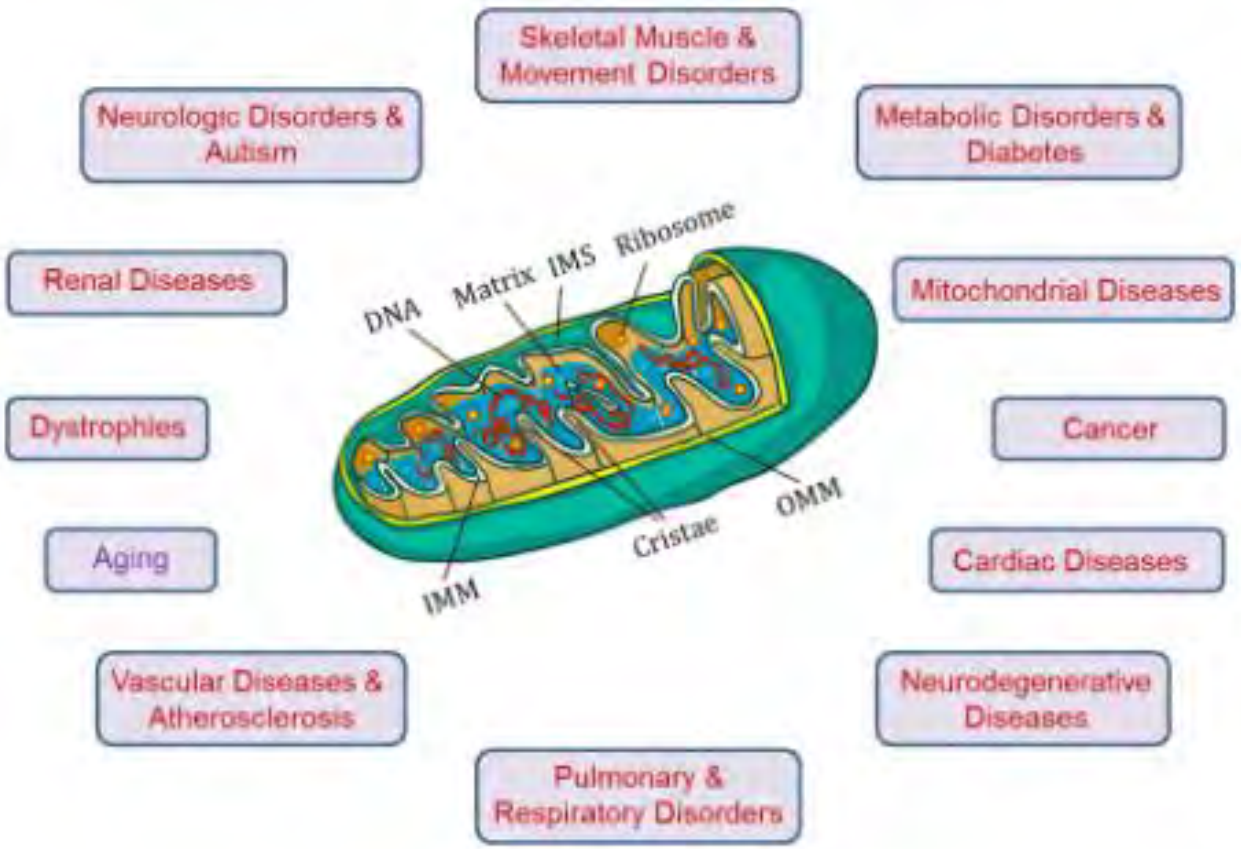
Laser energy fuels mitochondria

What time is best?

Low-level laser therapy – reported to increase mitochondrial function and ATP levels more effectively in the mornings, compared to afternoons or at night

A Valverde, C Hamilton, C Moro, et al. **Lights at night: does photobiomodulation improve sleep?**. *Neural Regeneration Research,* March 2023;18(3):474-77

What diseases are affected by the mitochondria?



Low-level laser therapy in Russia: history, science, and practice

Key takeaway:

If you combine laser and LED together, you get diminished results

Moskvin SV. **Low-Level Laser Therapy in Russia: History, Science and Practice**. *J Lasers Med Sci*. 2017 Spring;8(2):56-65

Key theme

- Electro-magnetic transfer

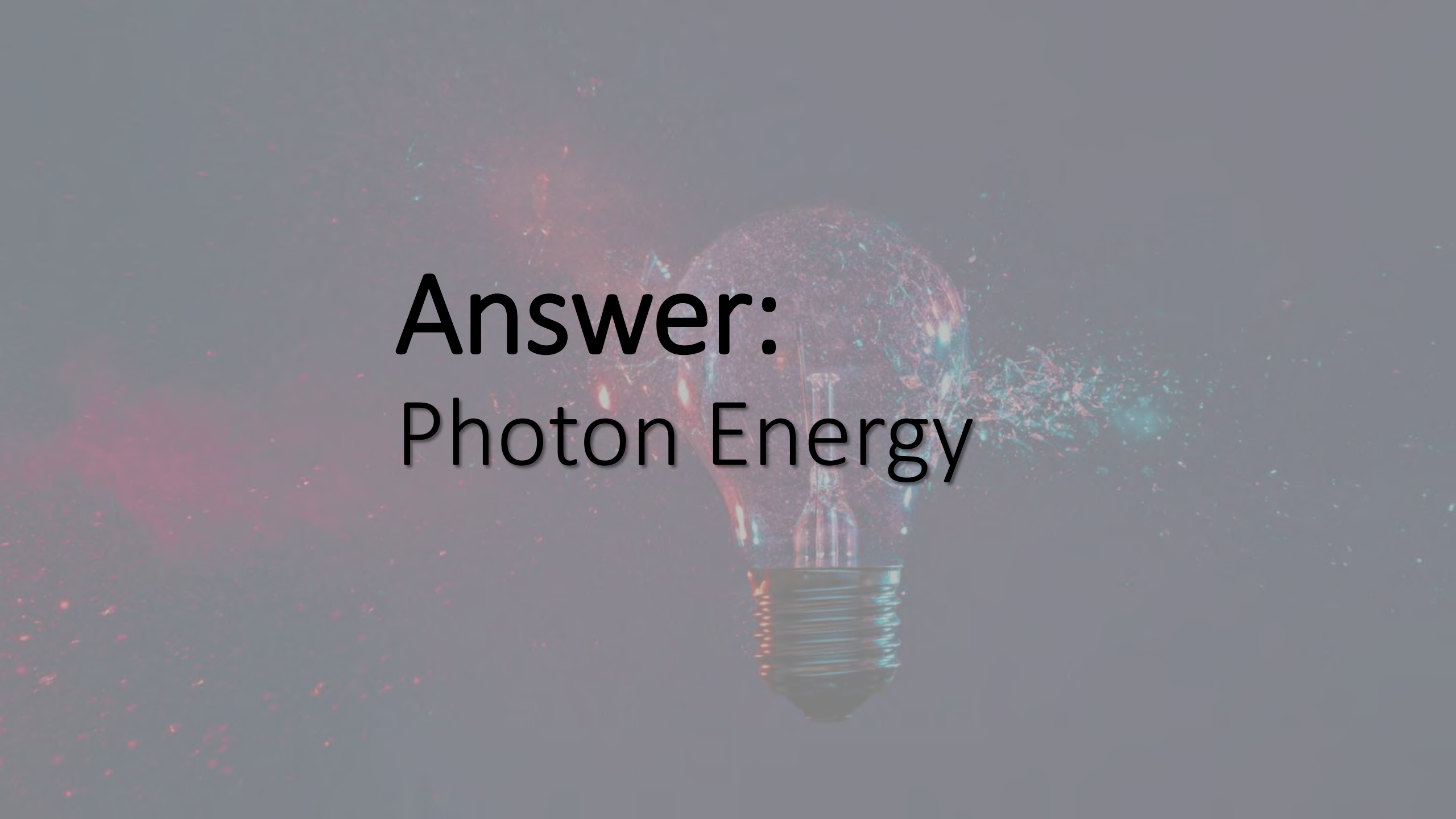
Factoid

8.2 billion photons per sec per diode

Electro-magnetic transfer

Wavelength		Energy
405 nm	98%	57% more than red
635 nm		27% more than 800 nm
800 nm		Baseline

Lower wavelength = higher energy



Answer:
Photon Energy

"To do good work, one must first have good tools"

~Confucius

Gvl

By Erchonia®

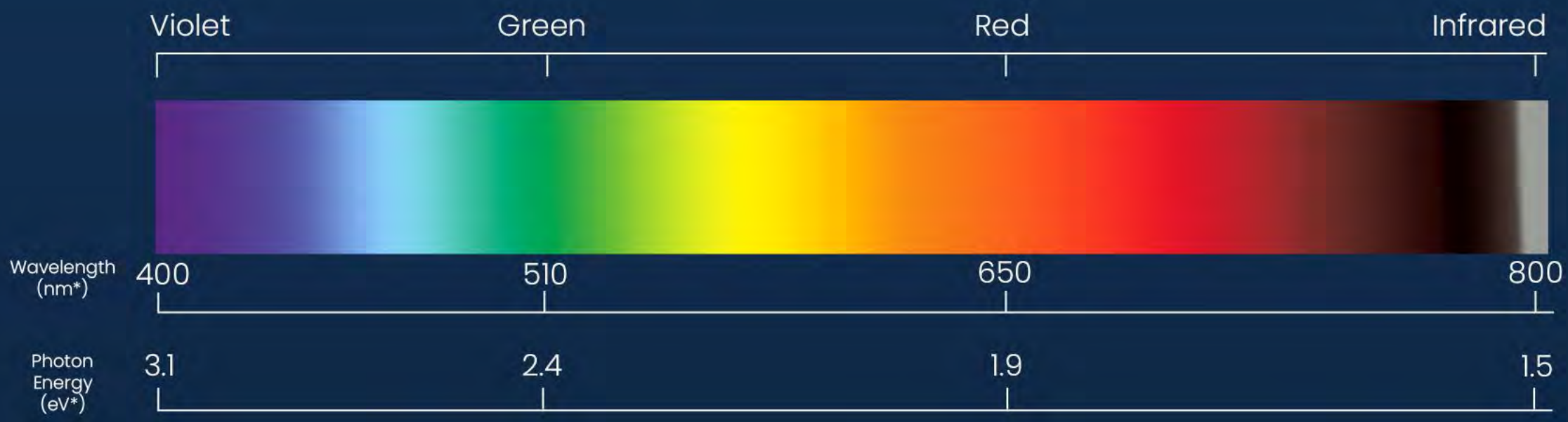
THE FIRST & ONLY GREEN & VIOLET LASER

FDA CLEARED SEPTEMBER 2022:
CHRONIC NECK AND SHOULDER PAIN

The most **energetic** laser in the world

Special Thanks to: Dr. Kirk Gair, Dr. Robert Silverman, Dr. Albert Comey

The most **energetic** laser in the world.



Photochemistry is dependent on the Photon Energy **(Electron Volts).. NOT POWER**

Key Concept: A minimum Photon Energy of 1.7 eV is required to cause electrons to jump to higher orbits. You can NOT make up for a lower eV by increasing the wattage (power) to trigger the same reactions.

GVL

By Erchonia®

PHOTON ENERGY IS ABSORBED BY THE MITOCHONDRIA

Mitochondria responsible for 90% of the ATP our body needs to function and has pivotal role in cell life and cell death

Mitochondria dysfunction common in:

- Long COVID
- Fatigue
- Aging
- Neurodegeneration
- Auto-immunity

GVL

By Erchonia®

Free floating mitochondria in bloodstream will absorb photon energy and disperse throughout body – allows photon energy to affect sites away from application site

Systemic or global effect

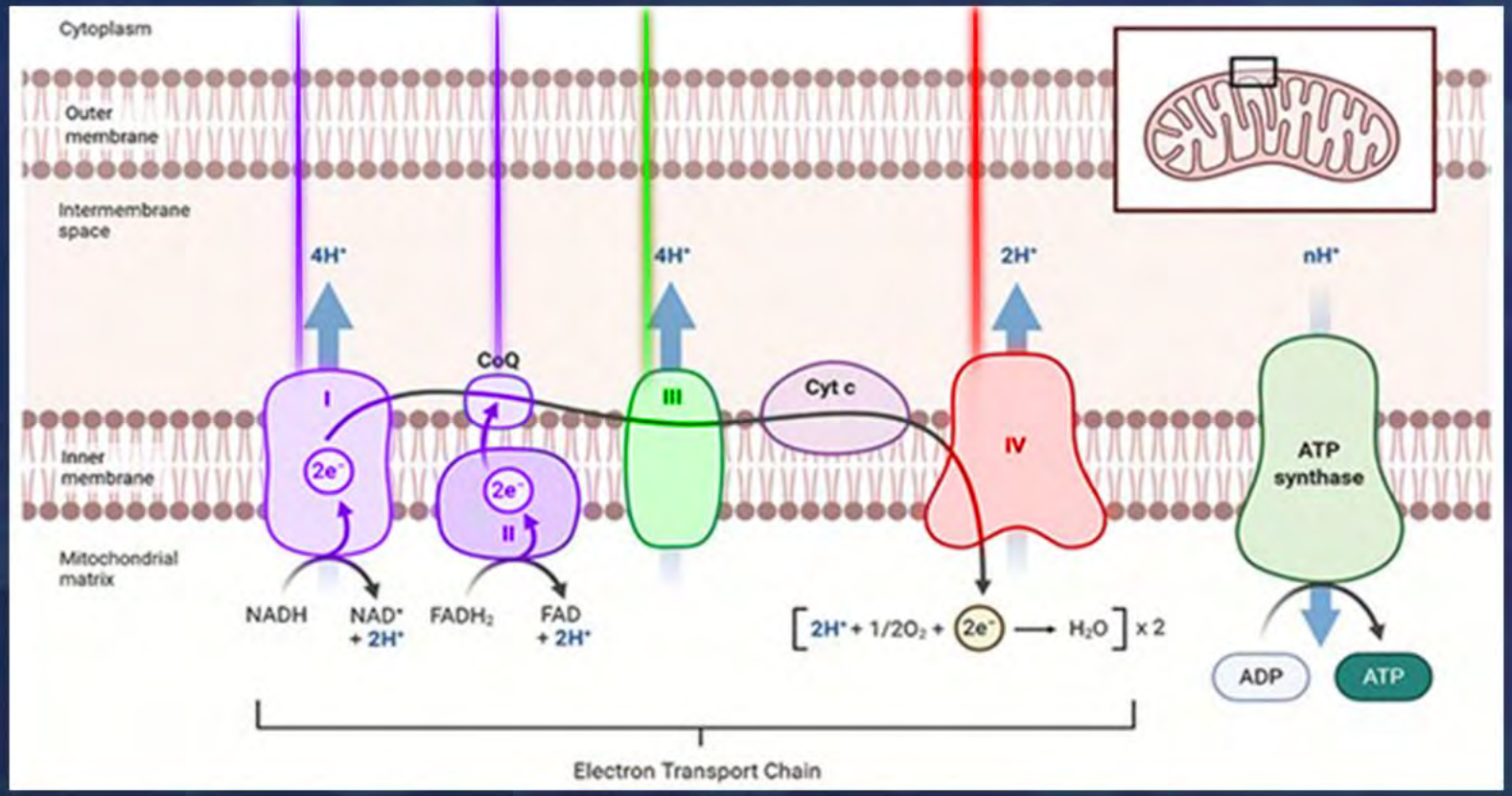
GVL

By Erchonia®

Different wavelengths of laser light can trigger biophotonic emissions from cells

Will travel through the microtubules and myelin sheaths like a fiber optic laser network

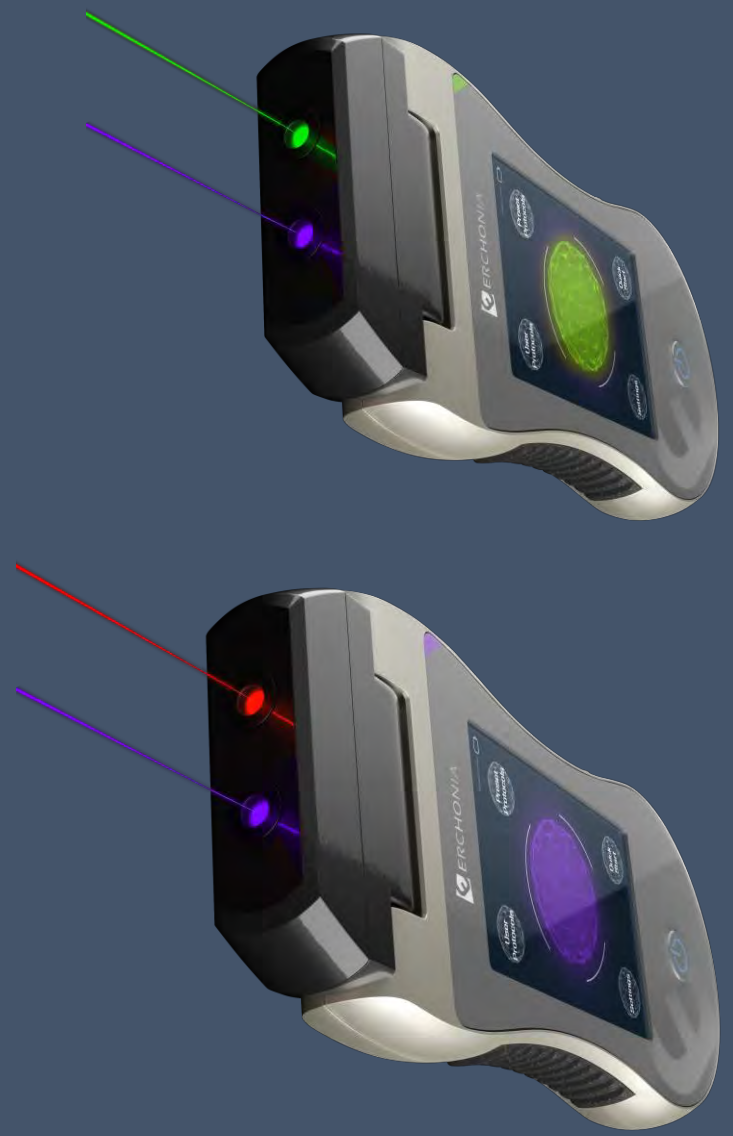
Mitochondria Electron Transport Chain

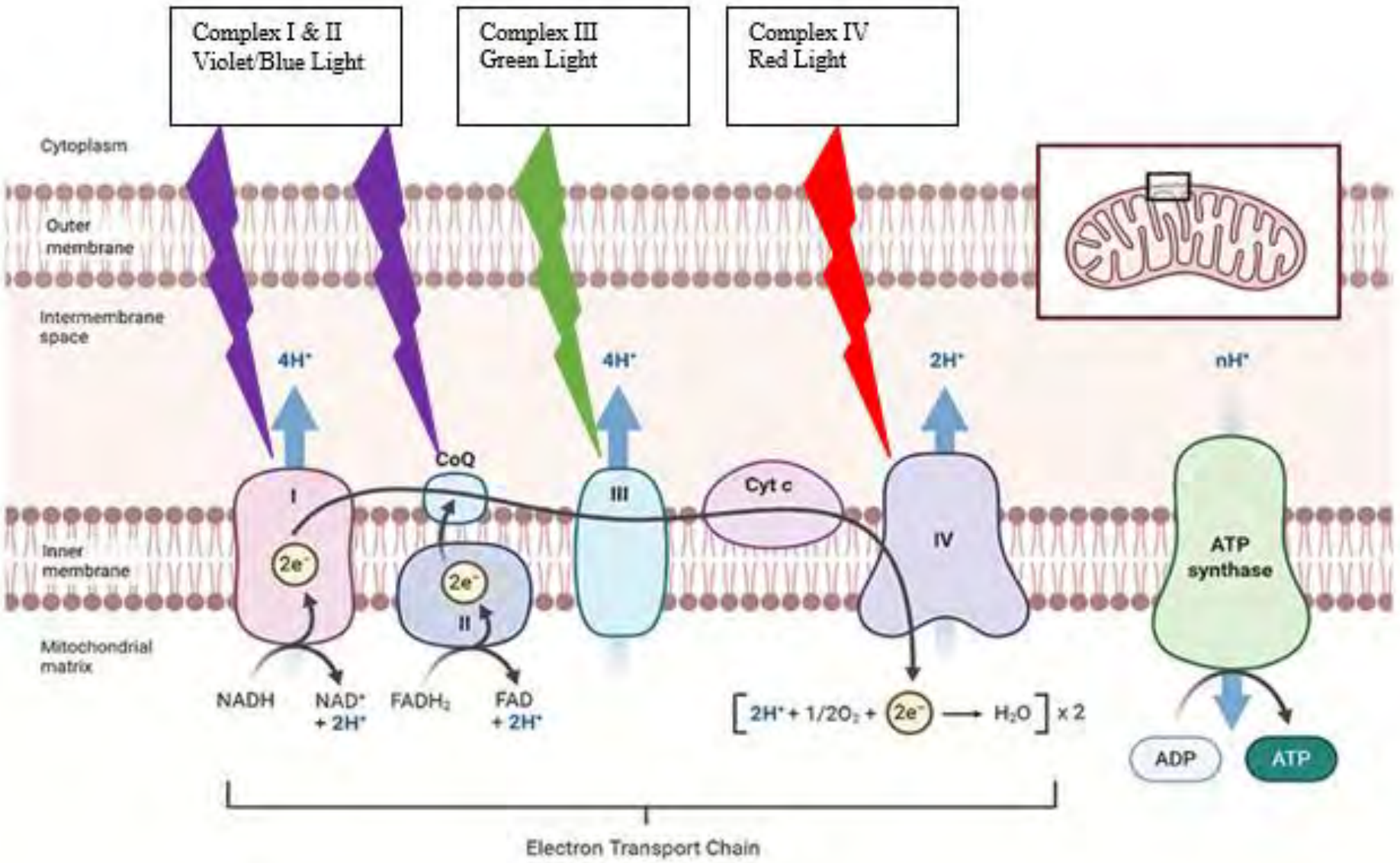


Photon Energy (Wavelength) required to excite each complex.

Complex 1&2	Complex 3	Complex 4
405nm	520nm	635nm

Treating the electron transport chain with Erchonia laser

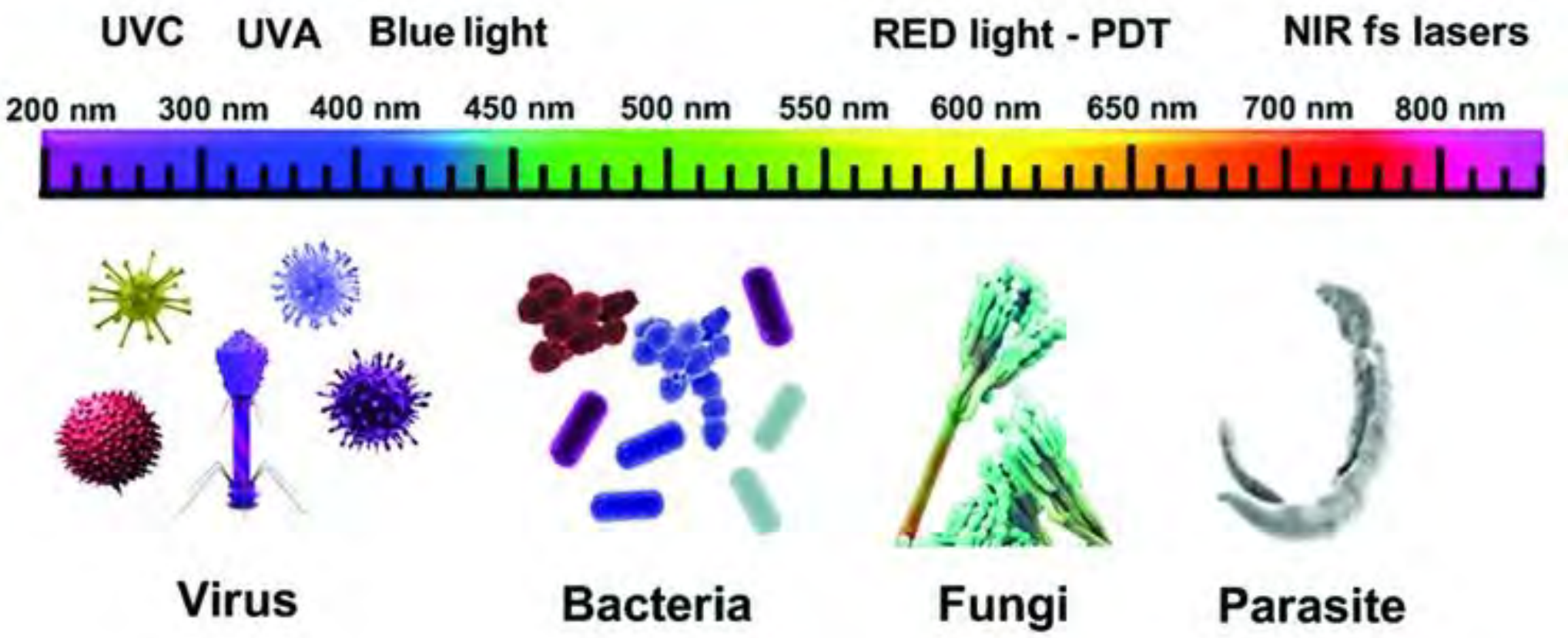



Complex I & II
Violet/Blue Light
Complex III
Green Light
Complex IV
Red Light
Cytoplasm
Outer membrane
Intermembrane space
Inner membrane
Mitochondrial matrix
4H+
4H+
2H+
nH+
CoQ
I
II
III
IV
Cyt c
2e-
2e-
ATP synthase
NADH
NAD+ + 2H+
FADH2
FAD + 2H+
[2H+ + 1/2O2 + 2e- → H2O] x 2
ADP
ATP
Electron Transport Chain

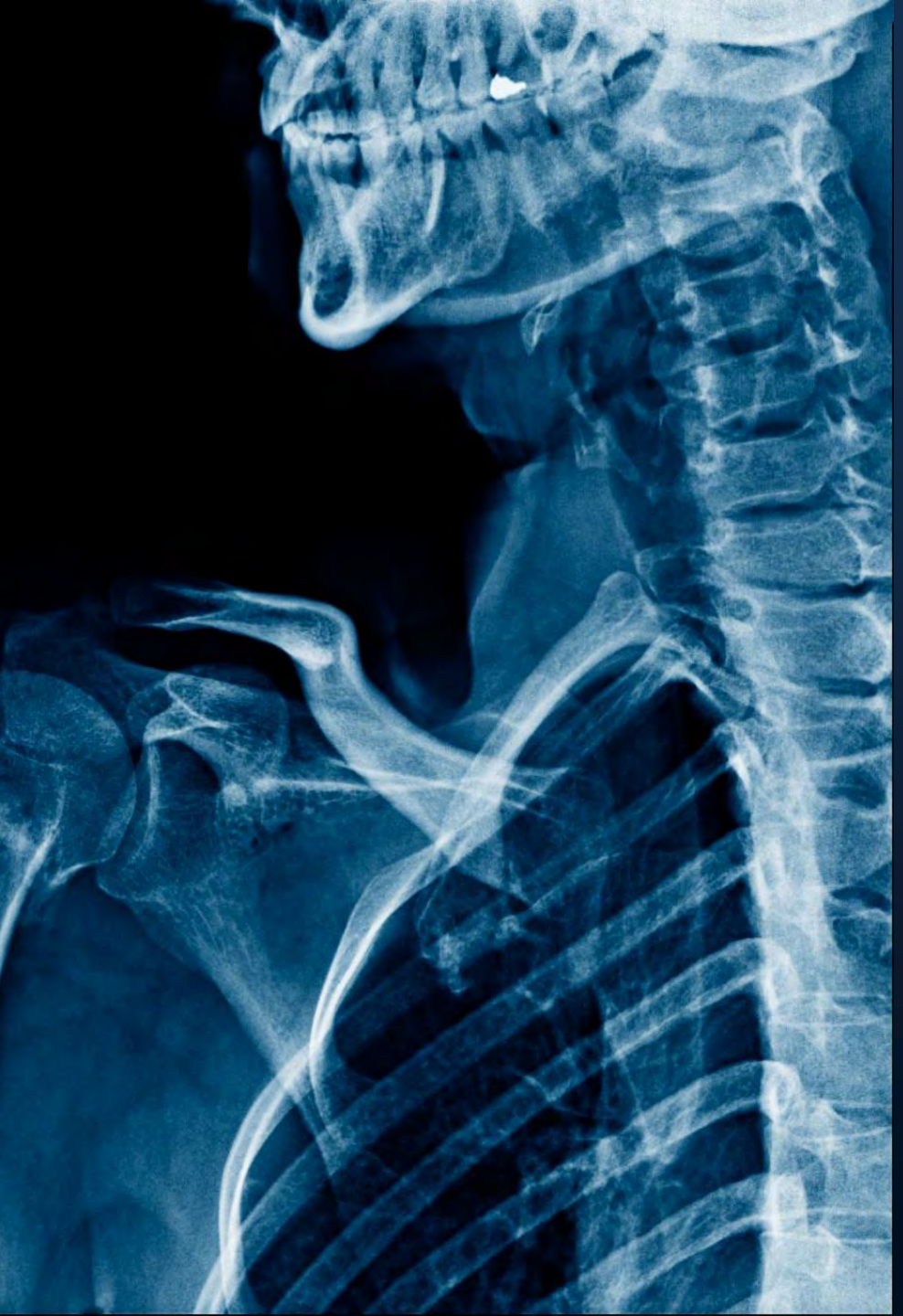
Key themes

- Complex I – considered “rate limiting reaction”
- Dysfunction – will affect all downstream complexes
- Complex I & II require violet wavelength as it supplies sufficient photonic energy to trigger needed electron jumps
- Complex III required to allow ETC to move into complex IV – green light is required
- Complex IV – only impacted by red wavelengths

Electromagnetic spectrum and its physiological effects on various microorganisms



Can biowarfare agents be defeated with light?. *Virulence*. 2013;4(8):796-825



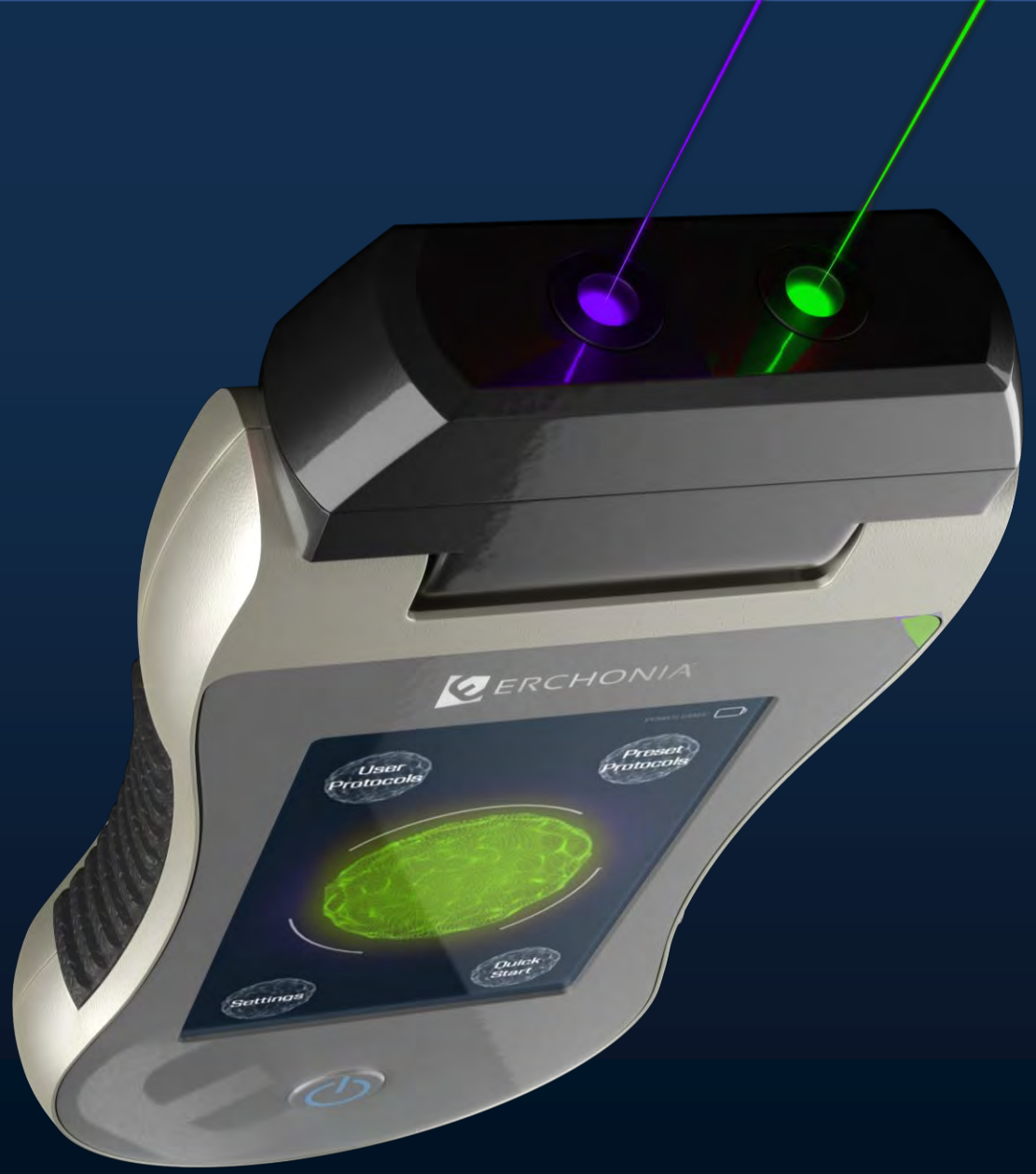
GVL

By Erchonia®

FDA Clinical Trial

Neck & shoulder PAIN

	Red Only	Red & Violet	Green & Violet
Subjects (n)	N=43	N=44	N=43
Duration of pain (months)	61.7	76.58	89.19
Subjects meeting study success criteria, ≥ 30% pain reduction	65%	75%	81%
(%) Improvement in Pain from baseline to immediately after treatment	48%	45%	52%
(%) Improvement in Pain from study endpoint to 48 hrs. post-treatment	43%	50%	65%
(°) Improvement in Range of Motion	14°	29°	32°

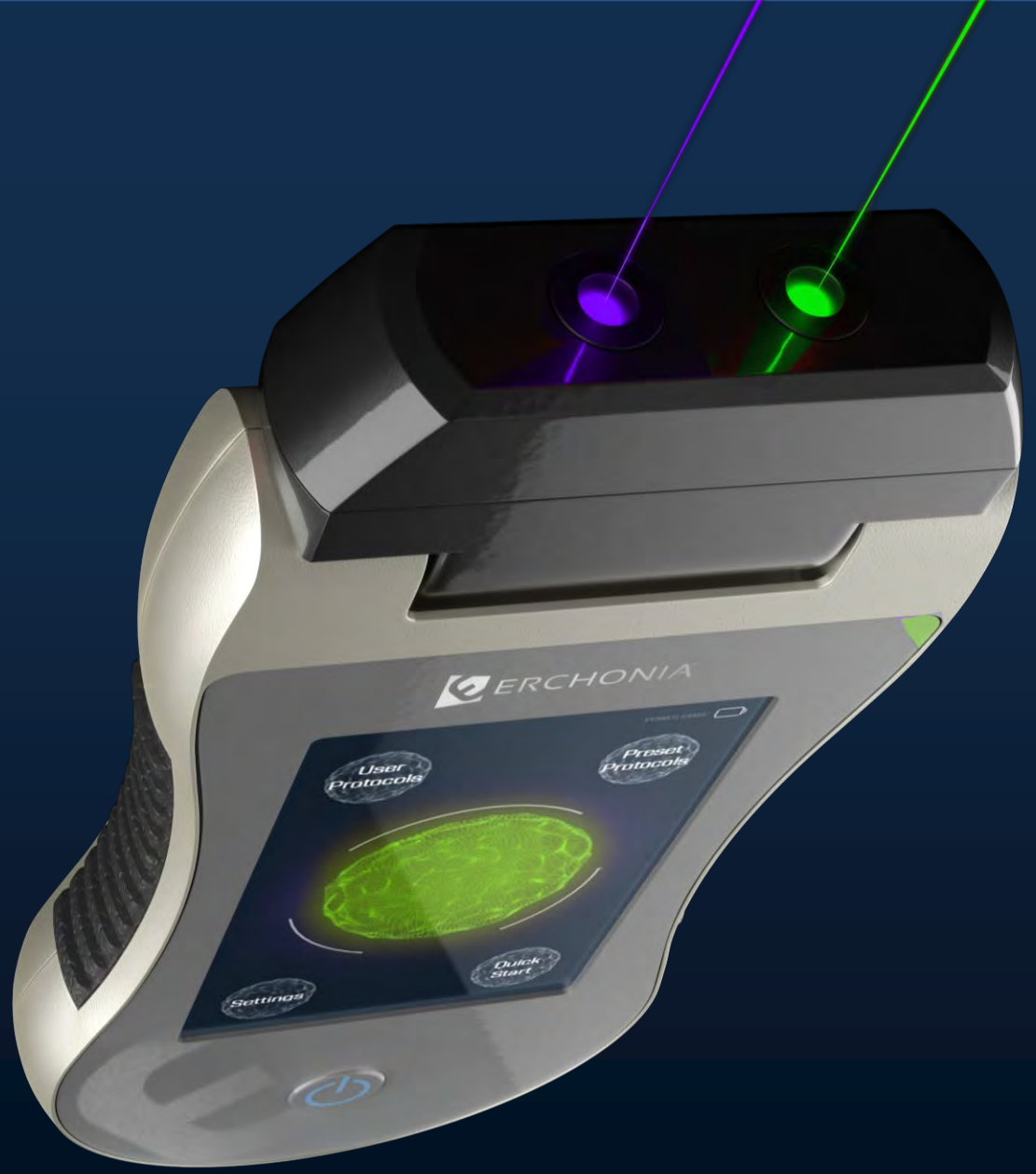


GVL

By Erchonia®

98% of subjects recorded they were Satisfied with treatment outcomes

100% of subjects recorded pain reduction at 24 hours post treatment



GVL

By Erchonia®

Case studies

Follow-up GVL – 6 months

U.S. FOOD & DRUG ADMINISTRATION

September 1, 2022

Erchonia Corporation
Travis Sammons
Contact Address

Re: K221987
Trade/Device Name: Erchonia® GVL
Regulation Number: 21 CFR 890.5500
Regulation Name: Infrared Lamp
Regulatory Class: Class II
Product Code: NHN, GEX
Dated: June 30, 2022
Received: July 6, 2022

Dear Travis Sammons:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. Although this letter refers to your product as a device, please be aware that some cleared products may instead be combination products. The 510(k) Premarket Notification Database located at https://www.accessdata.fda.gov/scripts/cdrh/cfdocs/cfpmn/pmn.cfm identifies combination product submissions. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Part 801); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803) for

Erchonia® GVL LASER
INDICATIONS FOR USE

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: 06/30/2023
See PRA Statement below.

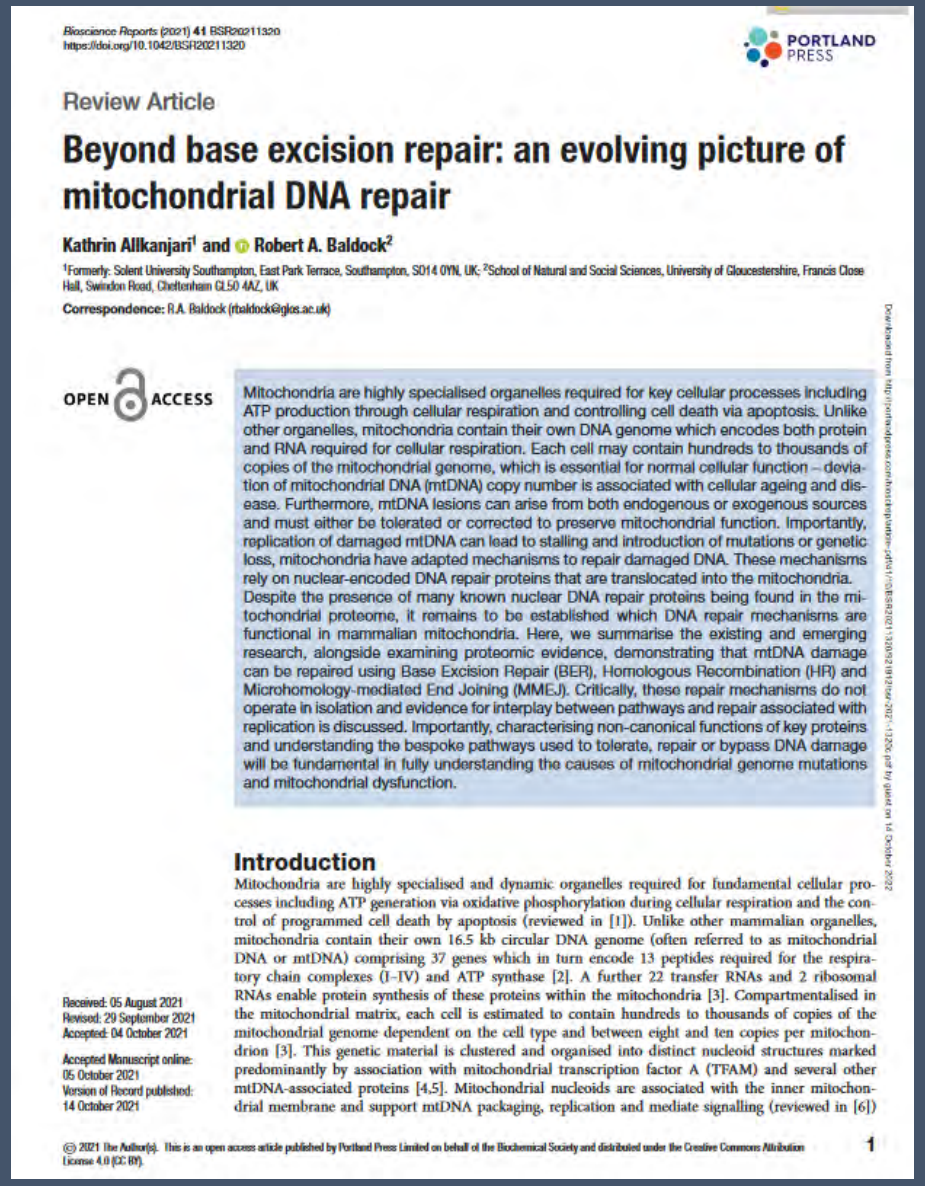
510(k) Number *(if known)*
K221987

Device Name
Erchonia® GVL

Indications for Use *(Describe)*
The Erchonia® GVL laser is generally indicated:
a. while using the green and violet diode simultaneously, for adjunctive use in providing temporary relief of minor chronic neck and shoulder pain of musculoskeletal origin,
b. and while using the violet diode, to treat dermatological conditions, and specifically indicated to treat moderate inflammatory Acne Vulgaris.

Vicious circle of mitochondria dysfunction

- Mitochondria contain own DNA (mtDNA)
- Replication of damaged mtDNA can lead to stalling and introduction of mutations or genetic loss
- Dysfunctional mitochondria survive longer than healthy mitochondria



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PORTLAND PRESS

Review Article

Beyond base excision repair: an evolving picture of mitochondrial DNA repair

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OPEN ACCESS

Mitochondria are highly specialised organelles required for key cellular processes including ATP production through cellular respiration and controlling cell death via apoptosis. Unlike other organelles, mitochondria contain their own DNA genome which encodes both protein and RNA required for cellular respiration. Each cell may contain hundreds to thousands of copies of the mitochondrial genome, which is essential for normal cellular function – deviation of mitochondrial DNA (mtDNA) copy number is associated with cellular ageing and disease. Furthermore, mtDNA lesions can arise from both endogenous or exogenous sources and must either be tolerated or corrected to preserve mitochondrial function. Importantly, replication of damaged mtDNA can lead to stalling and introduction of mutations or genetic loss, mitochondria have adapted mechanisms to repair damaged DNA. These mechanisms rely on nuclear-encoded DNA repair proteins that are translocated into the mitochondria. Despite the presence of many known nuclear DNA repair proteins being found in the mitochondrial proteome, it remains to be established which DNA repair mechanisms are functional in mammalian mitochondria. Here, we summarise the existing and emerging research, alongside examining proteomic evidence, demonstrating that mtDNA damage can be repaired using Base Excision Repair (BER), Homologous Recombination (HR) and Microhomology-mediated End Joining (MMEJ). Critically, these repair mechanisms do not operate in isolation and evidence for interplay between pathways and repair associated with replication is discussed. Importantly, characterising non-canonical functions of key proteins and understanding the bespoke pathways used to tolerate, repair or bypass DNA damage will be fundamental in fully understanding the causes of mitochondrial genome mutations and mitochondrial dysfunction.

Introduction

Mitochondria are highly specialised and dynamic organelles required for fundamental cellular processes including ATP generation via oxidative phosphorylation during cellular respiration and the control of programmed cell death by apoptosis (reviewed in [1]). Unlike other mammalian organelles, mitochondria contain their own 16.5 kb circular DNA genome (often referred to as mitochondrial DNA or mtDNA) comprising 37 genes which in turn encode 13 peptides required for the respiratory chain complexes (I–IV) and ATP synthase [2]. A further 22 transfer RNAs and 2 ribosomal RNAs enable protein synthesis of these proteins within the mitochondria [3]. Compartmentalised in the mitochondrial matrix, each cell is estimated to contain hundreds to thousands of copies of the mitochondrial genome dependent on the cell type and between eight and ten copies per mitochondrion [3]. This genetic material is clustered and organised into distinct nucleoid structures marked predominantly by association with mitochondrial transcription factor A (TFAM) and several other mtDNA-associated proteins [4,5]. Mitochondrial nucleoids are associated with the inner mitochondrial membrane and support mtDNA packaging, replication and mediate signalling (reviewed in [6])

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Version of Record published: 14 October 2021

Neurodegenerative diseases

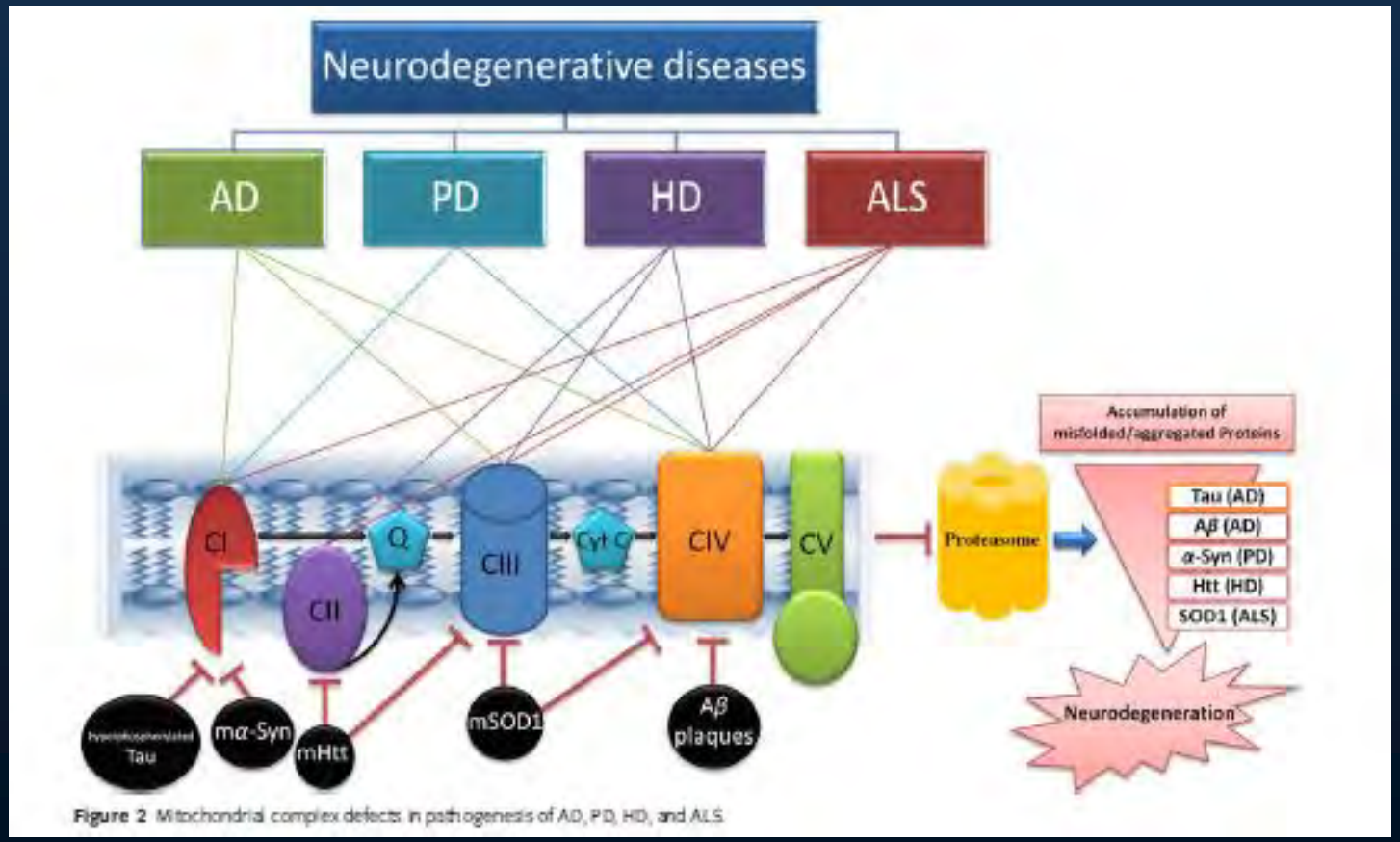

Figure 2 Mitochondrial complex defects in pathogenesis of AD, PD, HD, and ALS.

CNS Neuroscience & Therapeutics

REVIEW

CNS Neuroscience & Therapeutics

Mitochondrial Dysfunction and Biogenesis in Neurodegenerative diseases: Pathogenesis and Treatment

Mojtaba Golpich,[1] Elham Amini,[1] Zahurin Mohamed,[2] Raymond Azman Ali,[1] Norlinah Mohamed Ibrahim[1] & Abolhassan Ahmadiani[3]

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Keywords
Mitochondrial Biogenesis; Mitochondrial complexes; Mitochondrial Dysfunction; Neurodegenerative Diseases; Pathogenesis; Treatment.

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The first two authors contributed equally to this work.

SUMMARY

Neurodegenerative diseases are a heterogeneous group of disorders that are incurable and characterized by the progressive degeneration of the function and structure of the central nervous system (CNS) for reasons that are not yet understood. Neurodegeneration is the umbrella term for the progressive death of nerve cells and loss of brain tissue. Because of their high energy requirements, neurons are especially vulnerable to injury and death from dysfunctional mitochondria. Widespread damage to mitochondria causes cells to die because they can no longer produce enough energy. Several lines of pathological and physiological evidence reveal that impaired mitochondrial function and dynamics play crucial roles in aging and pathogenesis of neurodegenerative diseases. As mitochondria are the major intracellular organelles that regulate both cell survival and death, they are highly considered as a potential target for pharmacological-based therapies. The purpose of this review was to present the current status of our knowledge and understanding of the involvement of mitochondrial dysfunction in pathogenesis of neurodegenerative diseases including Alzheimer's disease (AD), Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS) and the importance of mitochondrial biogenesis as a potential novel therapeutic target for their treatment. Likewise, we highlight a concise overview of the key roles of mitochondrial electron transport chain (ETC.) complexes as well as mitochondrial biogenesis regulators regarding those diseases.

Introduction

Mitochondria play important roles in cell respiratory processes, metabolism, energy production, intracellular signaling, free radical production, and apoptosis [1,2]. These super dynamic organelles can change their morphology, number and function in reaction to physiological situations, and stressors like hormones, diet, temperature, and exercise [2]. Many lines of evidence suggest that mitochondria can critically regulate cell death and survival, play an essential role in aging, and are one of the key features of neurodegeneration [3]. In the central nervous system (CNS), sufficient energy supply which required for neuronal survival and excitability is mostly dependent on mitochondrial sources; therefore, brain is much more vulnerable to mitochondrial dysfunction [4]. Appropriate function of mitochondria is fundamental for activation of proper stress reactions and maintenance of metabolic homeostasis that have been implicated in life span extension and aging [5]. Cellular programs do the task of maintenance of mitochondrial quality and integrity by monitoring and substituting dysfunctional mitochondria with new

Neurodegenerative diseases

Violet impacts complex I – impacts tau and alpha-synuclein

Violet impacts complex II – impacts mHTT

Green impacts complex III – impacts mSOD1

Red impacts complex IV – impacts beta-amyloid plaques

Osteoarthritis

Recent ex vivo studies have reported mitochondrial dysfunction in human OA chondrocytes, and analyses of mitochondrial electron transport chain activity in these cells show decreased activity of Complexes I, II and III compared to normal chondrocytes.

Review > Nat Rev Rheumatol. 2011 Mar;7(3):161-9. doi: 10.1038/nrrheum.2010.213. Epub 2011 Jan 4.

The role of mitochondria in osteoarthritis

Francisco J Blanco [1], Ignacio Rego, Cristina Ruiz-Romero

Affiliations + expand

PMID: 21200395 DOI: 10.1038/nrrheum.2010.213

Abstract

Mitochondria are important regulators of cellular function and survival that may have a key role in aging-related diseases. Mitochondrial DNA (mtDNA) mutations and oxidative stresses are known to contribute to aging-related changes. Osteoarthritis (OA) is an aging-associated rheumatic disease characterized by articular cartilage degradation and elevated chondrocyte mortality. Articular cartilage chondrocytes survive and maintain tissue integrity in an avascular, low-oxygen environment. Recent ex vivo studies have reported mitochondrial dysfunction in human OA chondrocytes, and analyses of mitochondrial electron transport chain activity in these cells show decreased activity of Complexes I, II and III compared to normal chondrocytes. This mitochondrial dysfunction may affect several pathways that have been implicated in cartilage degradation, including oxidative stress, defective chondrocyte biosynthesis and growth responses, increased cytokine-induced chondrocyte inflammation and matrix catabolism, cartilage matrix calcification, and increased chondrocyte apoptosis. Mitochondrial dysfunction in OA chondrocytes may derive from somatic mutations in the mtDNA or from the direct effects of proinflammatory mediators such as cytokines, prostaglandins, reactive oxygen species and nitric oxide. Polymorphisms in mtDNA may become useful as biomarkers for the diagnosis and prognosis of OA, and modulation of serum biomarkers by mtDNA haplogroups supports the concept that mtDNA haplogroups may define specific OA phenotypes in the complex OA process.

Similar articles

Mitochondrial dysfunction in osteoarthritis.
Blanco FJ, López-Armada MJ, Maneiro E.
Mitochondrion. 2004 Sep;4(5-6):715-28. doi: 10.1016/j.mito.2004.07.022. Epub 2004 Oct 1.
PMID: 16120427

Mitochondrial DNA damage is involved in apoptosis caused by pro-inflammatory cytokines in human OA chondrocytes.
Kim J, Xu M, Xo R, Mates A, Wilson GL, Pearsall AW 4th, Grishko V.
Osteoarthritis Cartilage. 2010 Mar;18(3):424-32. doi: 10.1016/j.joca.2009.09.008. Epub 2009 Oct 1.
PMID: 19822235

1 common example that can inhibit the electron transport chain (etc) and impair mitochondrial function…

- *Common pain relievers like*
- *NSAIDs, aspirin, indomethacin, diclofenac, piroxicam and ibuprofen*

Common pain relievers/complex I

Major objective of present study – investigate whether in vitro the NSAIDs, aspirin, indomethacin, diclofenac, piroxicam and ibuprofen, which feature different chemical structures, able to inhibit mitochondrial complex I

› Chem Biol Interact. 2012 Jul 30;199(1):18-28. doi: 10.1016/j.cbi.2012.05.006. Epub 2012 May 28.

Inhibition of mitochondrial complex I by various non-steroidal anti-inflammatory drugs and its protection by quercetin via a coenzyme Q-like action

Cristian Sandoval-Acuña [1], Camilo Lopez-Alarcón, Margarita E Aliaga, Hernán Speisky

Affiliations + expand

PMID: 22652335 DOI: 10.1016/j.cbi.2012.05.006

Abstract

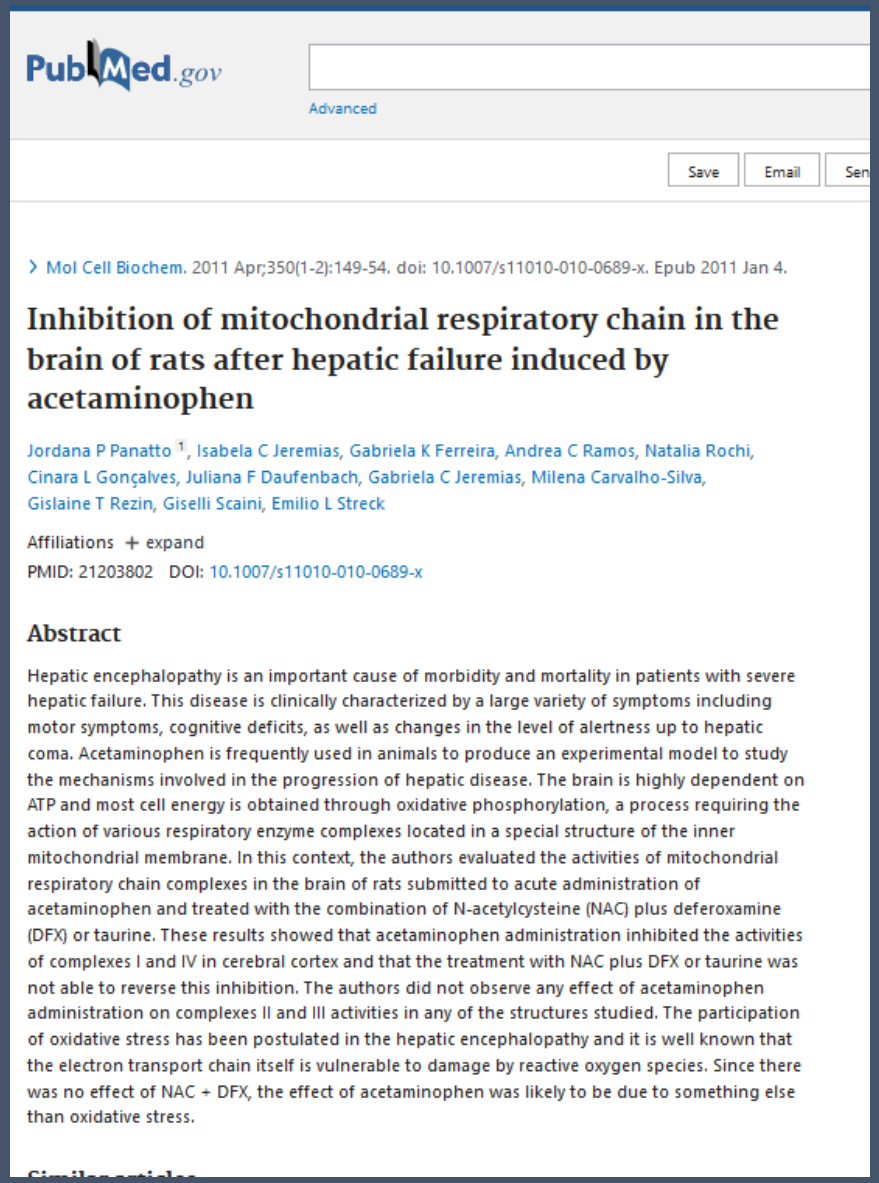
Mitochondrial dysfunction plays a major role in the development of oxidative stress and cytotoxicity induced by non-steroidal anti-inflammatory drugs (NSAIDs). A major objective of the present study was to investigate whether in vitro the NSAIDs, aspirin, indomethacin, diclofenac, piroxicam and ibuprofen, which feature different chemical structures, are able to inhibit mitochondrial complex I. All NSAIDs were effective inhibitors when added both, directly to mitochondria isolated from rat duodenum epithelium (50 μM) or to Caco-2 cells (250 μM). In the former system, complex I inhibition was concentration-dependent and susceptible to competition and reversion by the addition of coenzyme Q (32.5-520 μM). Based on reports suggesting a potential gastro-protective activity of quercetin, the ability of this flavonoid to protect isolated mitochondria against NSAIDs-induced complex I inhibition was evaluated. Low micromolar concentrations of quercetin (1-20 μM) protected against such inhibition, in a concentration dependent manner. In the case of aspirin, quercetin (5 μM) increased the IC50 by 10-fold. In addition, the present study shows that quercetin (5-10 μM) can behave as a "coenzyme Q-mimetic" molecule, allowing a normal electron flow along the whole electron transporting chain (complexes I, II, III and IV). The exposed findings reveal that complex I inhibition is a common deleterious effect of NSAIDs at the mitochondrial level, and that such effect is, for all tested agents, susceptible to be prevented by quercetin. Data provided here supports the contention that the protective action of quercetin resides on its, here for first time-shown, ability to behave as a coenzyme Q-like molecule.

Similar articles

Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit vascular smooth muscle cell proliferation via differential effects on the cell cycle.

Brooks G, Yu XM, Wang Y, Crabbe MJ, Shattock MJ, Harper JV.

Results: acetaminophen administration inhibited activities of complexes I and IV in cerebral cortex



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› Mol Cell Biochem. 2011 Apr;350(1-2):149-54. doi: 10.1007/s11010-010-0689-x. Epub 2011 Jan 4.

Inhibition of mitochondrial respiratory chain in the brain of rats after hepatic failure induced by acetaminophen

Jordana P Panatto [1], Isabela C Jeremias, Gabriela K Ferreira, Andrea C Ramos, Natalia Rochi, Cinara L Gonçalves, Juliana F Daufenbach, Gabriela C Jeremias, Milena Carvalho-Silva, Gislaine T Rezin, Giselli Scaini, Emilio L Streck

Affiliations + expand

PMID: 21203802 DOI: 10.1007/s11010-010-0689-x

Abstract

Hepatic encephalopathy is an important cause of morbidity and mortality in patients with severe hepatic failure. This disease is clinically characterized by a large variety of symptoms including motor symptoms, cognitive deficits, as well as changes in the level of alertness up to hepatic coma. Acetaminophen is frequently used in animals to produce an experimental model to study the mechanisms involved in the progression of hepatic disease. The brain is highly dependent on ATP and most cell energy is obtained through oxidative phosphorylation, a process requiring the action of various respiratory enzyme complexes located in a special structure of the inner mitochondrial membrane. In this context, the authors evaluated the activities of mitochondrial respiratory chain complexes in the brain of rats submitted to acute administration of acetaminophen and treated with the combination of N-acetylcysteine (NAC) plus deferoxamine (DFX) or taurine. These results showed that acetaminophen administration inhibited the activities of complexes I and IV in cerebral cortex and that the treatment with NAC plus DFX or taurine was not able to reverse this inhibition. The authors did not observe any effect of acetaminophen administration on complexes II and III activities in any of the structures studied. The participation of oxidative stress has been postulated in the hepatic encephalopathy and it is well known that the electron transport chain itself is vulnerable to damage by reactive oxygen species. Since there was no effect of NAC + DFX, the effect of acetaminophen was likely to be due to something else than oxidative stress.

Similar articles

Children Autism

In frontal cortex of Group A, lower level of ETC complexes observed in subset of autism cases:

- 60% (3/5) for complexes I, II, and IV
- 40% (2/5) for complexes III and IV

HHS Public Access
Author manuscript
J Neurochem. Author manuscript; available in PMC 2016 April 21.

Published in final edited form as:
J Neurochem. 2011 April ; 117(2): 209–220. doi:10.1111/j.1471-4159.2011.07189.x.

Brain region-specific deficit in mitochondrial electron transport chain complexes in children with autism

Abha Chauhan, **Feng Gu**, **Musthafa M. Essa**, **Jerzy Wegiel**, **Kulbir Kaur**, **William Ted Brown**, and **Ved Chauhan**
NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY, USA

Abstract

Mitochondria play important roles in generation of free radicals, ATP formation, and in apoptosis. We studied the levels of mitochondrial electron transport chain (ETC) complexes, that is, complexes I, II, III, IV, and V, in brain tissue samples from the cerebellum and the frontal, parietal, occipital, and temporal cortices of subjects with autism and age-matched control subjects. The subjects were divided into two groups according to their ages: Group A (children, ages 4–10 years) and Group B (adults, ages 14–39 years). In Group A, we observed significantly lower levels of complexes III and V in the cerebellum ($p < 0.05$), of complex I in the frontal cortex ($p < 0.05$), and of complexes II ($p < 0.01$), III ($p<0.01$), and V ($p < 0.05$) in the temporal cortex of children with autism as compared to age-matched control subjects, while none of the five ETC complexes was affected in the parietal and occipital cortices in subjects with autism. In the cerebellum and temporal cortex, no overlap was observed in the levels of these ETC complexes between subjects with autism and control subjects. In the frontal cortex of Group A, a lower level of ETC complexes was observed in a subset of autism cases, that is, 60% (3/5) for complexes I, II, and V, and 40% (2/5) for complexes III and IV. A striking observation was that the levels of ETC complexes were similar in adult subjects with autism and control subjects (Group B). A significant increase in the levels of lipid hydroperoxides, an oxidative stress marker, was also observed in the cerebellum and temporal cortex in the children with autism. These results suggest that the expression of ETC complexes is decreased in the cerebellum and the frontal and temporal regions of the brain in children with autism, which may lead to abnormal energy metabolism and oxidative stress. The deficits observed in the levels of ETC complexes in children with autism may readjust to normal levels by adulthood.

Keywords

autism; electron transport chain complexes; energy; mitochondria; oxidative stress

Address correspondence and reprint requests to Abha Chauhan, NYS Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314, USA. abha.chauhan@opwdd.ny.gov.

Supporting information

Additional supporting information may be found in the online version of this article:

Table S1. Case history of autism and control brain samples.

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Summary: strongest support for a role for complex I and/or IV deficits – pathophysiology of PD and AD

MDD	• Small number of studies with small sample sizes • Region-specific heterogeneity • **Moderate effects in complex I, small effects in complex IV**
BPD	• Small sample sizes • Region-specific heterogeneity • **Moderate effects in complex I, small effects in complex IV**
SZ	• Small sample sizes • Region-specific heterogeneity • **Moderate effects in complex I and IV**
AD	• Large number of studies with large sample sizes • Tissue-specific heterogeneity (low in blood, high in brain) • **Strong effects in complex I and IV**
PD	• Large number of studies • No heterogeneity • **Strong effects in complex I and IV**
AGING	• Most robust effects • No heterogeneity • **Strongest effects in complex I and IV**

Neuropsychopharmacology www.nature.com/npp

REVIEW ARTICLE

Multivariate meta-analyses of mitochondrial complex I and IV in major depressive disorder, bipolar disorder, schizophrenia, Alzheimer disease, and Parkinson disease

L Holper[1], D Ben-Shachar[2] and JJ Mann[1]

Complex I (NADH dehydrogenase, NDU) and complex IV (cytochrome c oxidase, COX) of the mitochondrial electron transport chain have been implicated in the pathophysiology of major psychiatric disorders, such as major depressive disorder (MDD), bipolar disorder (BD), and schizophrenia (SZ), as well as in neurodegenerative disorders, such as Alzheimer disease (AD) and Parkinson disease (PD). We conducted meta-analyses comparing complex I and IV in each disorder MDD, BD, SZ, AD, and PD, as well as in normal aging. The electronic databases Pubmed, EMBASE, CENTRAL, and Google Scholar, were searched for studies published between 1980 and 2018. Of 2049 screened studies, 125 articles were eligible for the meta-analyses. Complex I and IV were assessed in peripheral blood, muscle biopsy, or postmortem brain at the level of enzyme activity or subunits. Separate meta-analyses of mood disorder studies, MDD and BD, revealed moderate effect sizes for similar abnormality patterns in the expression of complex I with SZ in frontal cortex, cerebellum and striatum, whereas evidence for complex IV alterations was low. By contrast, the neurodegenerative disorders, AD and PD, showed strong effect sizes for shared deficits in complex I and IV, such as in peripheral blood, frontal cortex, cerebellum, and substantia nigra. Beyond the diseased state, there was an age-related robust decline in both complexes I and IV. In summary, the strongest support for a role for complex I and/or IV deficits, is in the pathophysiology of PD and AD, and evidence is less robust for MDD, BD, or SZ.

Neuropsychopharmacology (2019) 44:837–849; https://doi.org/10.1038/s41386-018-0090-0

INTRODUCTION

Mitochondrial dysfunction is implicated in the pathophysiology of major psychiatric disorders, such as major depressive disorder (MDD) [1], bipolar disorder (BD) [2] and schizophrenia (SZ) [3], as well as neurodegenerative disorders, such as Alzheimer disease (AD) [4] and Parkinson disease (PD) [4]. Mitochondria are intracellular organelles that produce adenosine triphosphate (ATP), the main source of cellular energy. Impaired mitochondrial function results in decreased ATP production, impaired bioenergetics, apoptosis and oxidative stress [5]. Prior to the generation of ATP, the electrons extracted from nutrients are transported along the electron transport chain (ETC) and the energy released is directed into a transmembrane proton.

Research has identified two enzymes of the ETC located at the inner mitochondrial membrane as being particular impaired in these five disorders MDD, BD, SZ, AD, and PD. The first enzyme, complex I (NADH dehydrogenase, NDU) consists of 45 subunits, seven of which are encoded by mitochondrial DNA (mtDNA) and the remaining subunits by nuclear DNA (nDNA). Complex I is one of the entry enzymes of cellular respiration or oxidative phosphorylation in the mitochondrion. It is also the largest multimeric enzyme complex of the ETC and is a major contributor to the generation of the proton gradient across the mitochondrial inner membrane, which drives ATP production. The second enzyme of interest, complex IV (cytochrome-c-oxidase, COX) consists of 13 subunits, three of which are encoded by mtDNA, the remainder by nDNA. Complex IV catalyzes the final step in the mitochondrial ETC and, due to its rate-limiting role in this oxidative process [6], has been proposed as a key markers of mitochondrial function [7]. Numerous excellent reviews [1–4] have discussed the details of impairments in both complex I and IV enzyme activities and subunit assembly within each of the above mentioned disorders. A meta-analysis summarizing the findings across these disorders could not be found in the literature. The remaining complexes II (succinate dehydrogenase), III (cytochrome c reductase) and V (ATP synthase) either have not been studied, or they have been studied to a much smaller degree in these five disorders, compared with complex I and IV, and thus there are too few data for a meta-analysis.

We chose these five disorders MDD, BD, SZ, AD, and PD not only because of the potential common mitochondrial dysfunction, but also based on their clinical similarities. Though regarded as different disorders in major classification systems like DSM and ICD, there is also overlap in clinical symptoms. Depression is found in mood disorders, but is also frequent in schizophrenia and both AD and PD [8, 9]. Psychotic symptoms are observed in MDD and BD, as well as SZ ((DSM-IV-TR), [10]). Although, Alzheimer's and Parkinson's diseases have distinct brain histopathology, both are age-related neurodegenerative conditions characterized by memory loss and depression and have some commonality in molecular

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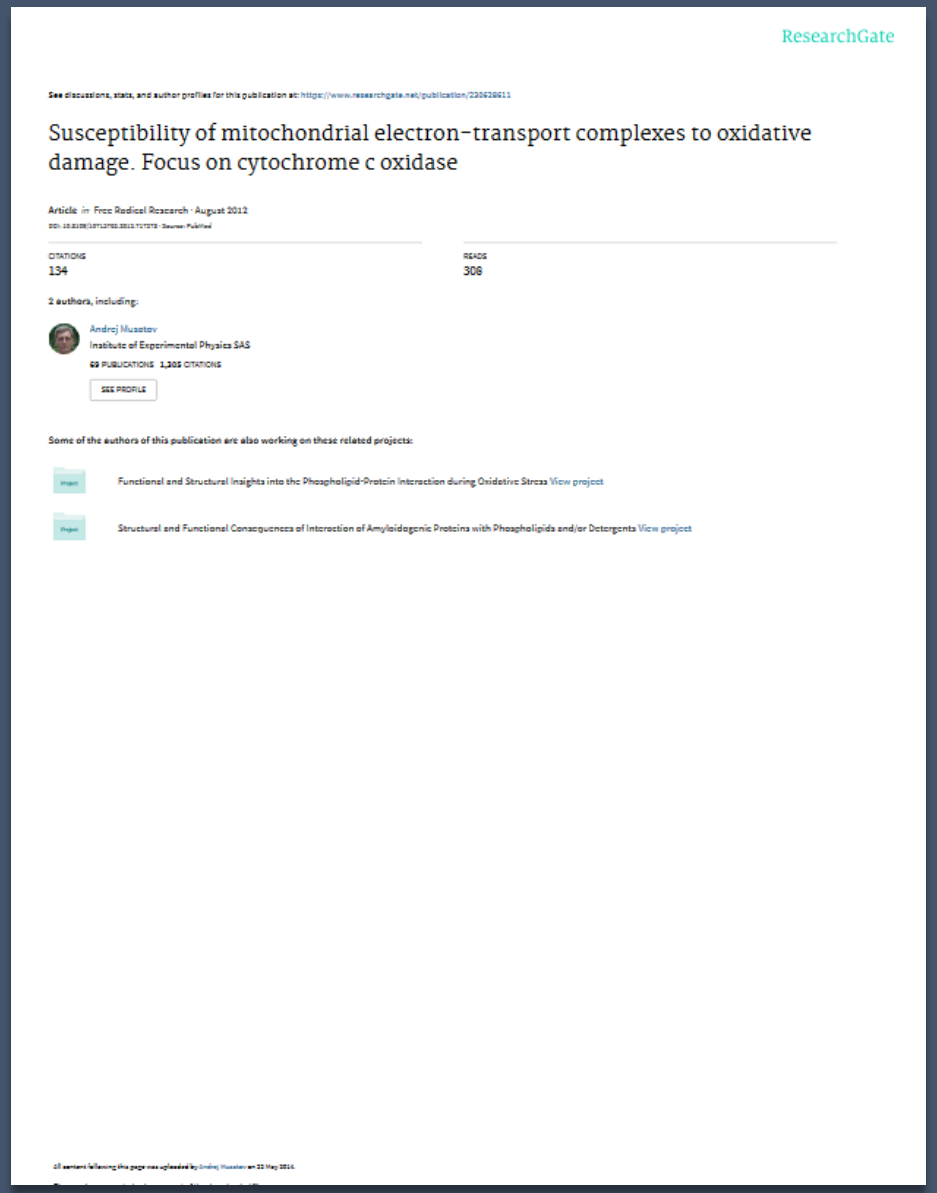
Correspondence: L Holper (holpersini.phys.ethz.ch)

Received: 7 March 2018 Revised: 26 April 2018 Accepted: 7 May 2018
Published online: 16 May 2018

Ischemia/reperfusion injury, Parkinson's, Alzheimer's, neurodegenerative diseases, other age-related degenerative changes

Two mitochondrial electron-transfer complexes are major sources of ROS: complex I and complex III. Oxidative damage to either of these expected to inhibit electron transport



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Susceptibility of mitochondrial electron-transport complexes to oxidative damage. Focus on cytochrome c oxidase

Article in Free Radical Research · August 2012

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Aging

Skeletal muscle:

- Statistically significant **decline in activities of complexes I, II and IV** of mitochondria isolated from the vastus lateralis muscle of 29 orthopaedic patients aged 20-90 years

Brain:

- Age-related effects on electron transport in brain tissue restricted primarily to complex I. Bowling et al. (1993) of crude mitochondrial preparations from the fronto-parietal cortex of rhesus monkeys showed **statistically significant decline in complex I activity relative** to that of citrate synthase and in complex IV, to lesser degree. Complex II–III activity not affected with age

Aging Cell (2004) 3, pp21–27 Doi: 10.1111/j.1474-9728.2003.00078.x

REVIEW

Is defective electron transport at the hub of aging?

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Summary

The bulwark of the mitochondrial theory of aging is that a defective respiratory chain initiates the death cascade. The increased production of superoxide is suggested to result in progressive oxidant damage to cellular components and particularly to mtDNA that encodes subunits assembled in respiratory complexes. Earlier studies of respiration in muscle mitochondria obtained from large cohorts of patients supported this notion by showing that either singly or in combinations, the respiratory complexes exhibited decreased activity in the elderly. The following critique of the most cited publications over the past decade points out the systematic errors that put earlier work at odds with recent findings. These later investigations indicate that aging has no overt effect on either the electron transport system or oxidative phosphorylation.

Key words: aging; electron transport; mitochondria; superoxide; tissues/species.

Introduction

Comparative studies with animals suggest that longer lifespans correlate best with low oxygen radical (ROS) production specifically by mitochondria (Perez-Campo *et al.*, 1998). Thus oxidant damage to mitochondria or other cell components provides the most widely believed mechanism for the aging process. Debate still centres on whether electron transport dysfunction is instrumental in the process or, instead, the cumulative damage to protein, lipid and DNA (Rustin *et al.*, 2000; Szibor & Holtz, 2003). A wealth of genetic and biochemical information exists to indicate that ROS are not only responsible for oxidative stress, but also act as secondary messengers by influencing the interplay of various signalling pathways involved in the decision by cells to undergo proliferation, senescence or apoptosis (Finkel & Holbrook, 2000; Hekimi & Guarente, 2003).

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The electron transport chain

A major source of cellular superoxide ($O_2^{\bullet-}$), which yields relatively stable peroxide (H_2O_2) by dismutation and the highly reactive hydroxyl ($^{\bullet}OH$) species by Fenton chemistry, is the respiratory chain located in the inner membrane of eukaryotic mitochondria and shown schematically in Fig. 1. In this energy transducing apparatus, electrons from NADH–ubiquinone oxidoreductase (complex I) are passed downhill via the ubiquinone (Q) pool and complex III (ubiquinone–cytochrome *c* oxidoreductase) to cytochrome oxidase (complex IV), where oxygen is reduced to water. Other major entry points for reducing equivalents into the chain are the three membrane-bound dehydrogenases succinate-Q oxidoreductase (complex II), ETF-Q oxidoreductase, and α-glycerolphosphate-Q oxidoreductase. These enzymes provide direct links to TCA cycle activity, fatty acid oxidation and glycolysis, respectively, and help gear activity to electron flow in the chain through competition for oxidized Q. An adequate supply of reducing equivalents is as critical to energy conservation as a properly functioning electron transport chain. Owing to pumping of protons into the intermembrane space by complexes I, III and IV during redox cycling, electron flow produces an electrochemical proton gradient ($\Delta\mu H^+$) across the inner membrane, which controls mitochondrial respiration and ATP synthesis by ATP synthase (complex V), ionic equilibria essential to mitochondrial stability and cell viability, and the production of $O_2^{\bullet-}$. For simplicity the subsets of reactions constituting each of these processes can be grouped into the modules shown in Fig. 2.

Metabolic control analysis applied to mitochondria has shown that control of respiration in state 3 (high ATP demand) is distributed between the respiratory chain and phosphorylation system; control in heart and muscle is mainly by the respiratory chain, whereas in brain, liver and kidney it is by the phosphorylation system. Hence, within limits, heart and muscle might be expected to be more sensitive to respiratory chain deficiencies than the other tissues (Rossignol *et al.*, 2000). In state 4 conditions (minimum ATP demand) respiration is controlled mainly by proton leak with some by substrate oxidation. Under physiological conditions mitochondrial function and thus $O_2^{\bullet-}$ production is altered by a variety of transient and long-term effectors, e.g. Ca^{2+} stimulates NADH delivery to the electron transport chain (Denton & McCormack, 1985); nitric oxide (Cooper, 2002) or phosphorylation (Lee *et al.*, 2002a) modulates complex IV; oxidized glutathione inhibits reversibly complex I (Taylor *et al.*, 2003) and α-ketglutarate dehydrogenase (Nulton-Persson *et al.*, 2003) to slow TCA cycle activity; glucagon stimulates complex II (Brand *et al.*, 1990), thyroxine modulates proton leak (Harper & Brand, 1993), and part of the anti-aging

Obesity

Western blot analyses revealed significantly **fewer** **complex I and IV** components in adipose tissues from obese compared with nonobese women.

Results suggest differences at level of respiratory chain complexes might be responsible for deterioration of respiratory capacity in obese individuals

Am J Physiol Endocrinol Metab 309: E380–E387, 2015.
First published June 16, 2015; doi:10.1152/ajpendo.00524.2014.

Inverse relationship between body mass index and mitochondrial oxidative phosphorylation capacity in human subcutaneous adipocytes

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[1]Technische Universität München, ZIEL Institute for Food and Health, Nutritional Medicine, Freising-Weihenstephan, Germany; [2]Technische Universität München, Molecular Nutritional Medicine, Freising-Weihenstephan, Germany; [3]Institute of Nutritional Medicine, Klinikum rechts der Isar, Technische Universität München, Munich, Germany; and [4]Technische Universität München, ZIEL, Molecular Nutritional Medicine, Freising-Weihenstephan, Germany

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Fischer B, Schöttl T, Schempp C, Fromme T, Hauner H, Klingenspor M, Skurk T. Inverse relationship between body mass index and mitochondrial oxidative phosphorylation capacity in human subcutaneous adipocytes. *Am J Physiol Endocrinol Metab* 309: E380–E387, 2015. First published June 16, 2015; doi:10.1152/ajpendo.00524.2014.—Obesity is characterized by a substantial increase in adipose tissue that may contribute to energy balance. Recently, obesity was suggested to be associated with impaired mitochondrial function in adipocytes. In this study, we investigated the following: *1*) the respiratory capacities of mitochondria isolated from mature adipocytes of female subjects whose body mass index (BMI) values were distributed over a wide range and 2) the amounts of electron transport chain complexes in these mitochondria. Fat cells were isolated from adipose tissue specimens by collagenase digestion. Mitochondria were isolated from these fat cells, and their respiratory capacity was determined using a Clark-type electrode. Fat cells were also sorted on the basis of their size into large and small fractions to assess their respiration. Western blot analyses were performed to quantify respiratory chain complex components. We also examined mitochondrial activity development during differentiation using human Simpson-Golabi-Behmel syndrome cells. Our results showed that mitochondrial respiratory capacities in adipocytes were inversely associated with BMI values but were independent of cell size. Western blot analyses revealed significantly fewer complex I and IV components in adipose tissues from obese compared with nonobese women. These results suggest that differences at the level of respiratory chain complexes might be responsible for the deterioration of respiratory capacity in obese individuals. In particular, electron transport at the level of complexes I and IV seems to be most affected.

obesity; adipocytes; mitochondrial respiration; respiratory chain complexes; oxidative phosphorylation

ADIPOSE TISSUE IS AN IMPORTANT CONTRIBUTOR to the regulation of energy homeostasis. Although adipose tissue accounts for only about 4% of whole body energy turnover in normal-weight individuals (12), its contribution might increase considerably due to the increased adipose tissue mass associated with increasing obesity. It is well accepted that obesity is accompanied by adipocyte dysregulation, which is linked to abnormal adipokine secretion, an inflammatory status of adipose tissue, and ultimately to metabolic disorders like type 2 diabetes mellitus (reviewed in Ref. 11). Altered mitochondrial function in white adipose tissue has also been considered to be involved in abnormal metabolic states, as seen in obesity (37, 38).

Thermogenesis by adipocytes from obese donors was found to be reduced compared with that by adipocytes from lean donors (4). Moreover, obesity was found to be associated with the downregulation of transcription levels of genes that were involved in oxidative phosphorylation (OXPHOS) in white adipose tissue (25). However, other investigators did not find a link between the degree of obesity and electron transport chain gene transcription levels in adipose tissue (9). Nevertheless, basal oxygen consumption per gram of adipose tissue was found to be higher in lean subjects than in obese subjects (12).

In line with this, the maximal respiration rates of mitochondria isolated from small and large adipocytes were negatively correlated with body mass index (BMI) values (38). Furthermore, in vitro-differentiated preadipocytes from human subcutaneous adipose tissue of obese donors had lower oxygen consumption rates after isoproterenol stimulation compared with those from lean donors (37). However, the correlation between mitochondrial respiration in mature adipocytes and BMI remains largely unknown. Moreover, previous research did not address different mitochondrial respiration states and BMI values for unilocular fat cells.

In this study, we examined different OXPHOS states and variables associated with respiratory control of mitochondria that were isolated from subcutaneous abdominal adipocytes from women whose BMI values were distributed over a wide range. We also characterized mitochondrial respiration on the basis of different adipocyte sizes in a subpopulation. Because it is largely unknown which factors account for the differences in the respiratory rate in obese individuals, we also compared the amounts of respiratory chain component proteins in adipocytes from lean and obese women. To acquire further insights into mitochondrial function during adipocyte differentiation, we evaluated the respiration of isolated mitochondria during Simpson-Golabi-Behmel syndrome (SGBS) cell differentiation, a human preadipocyte cell model.

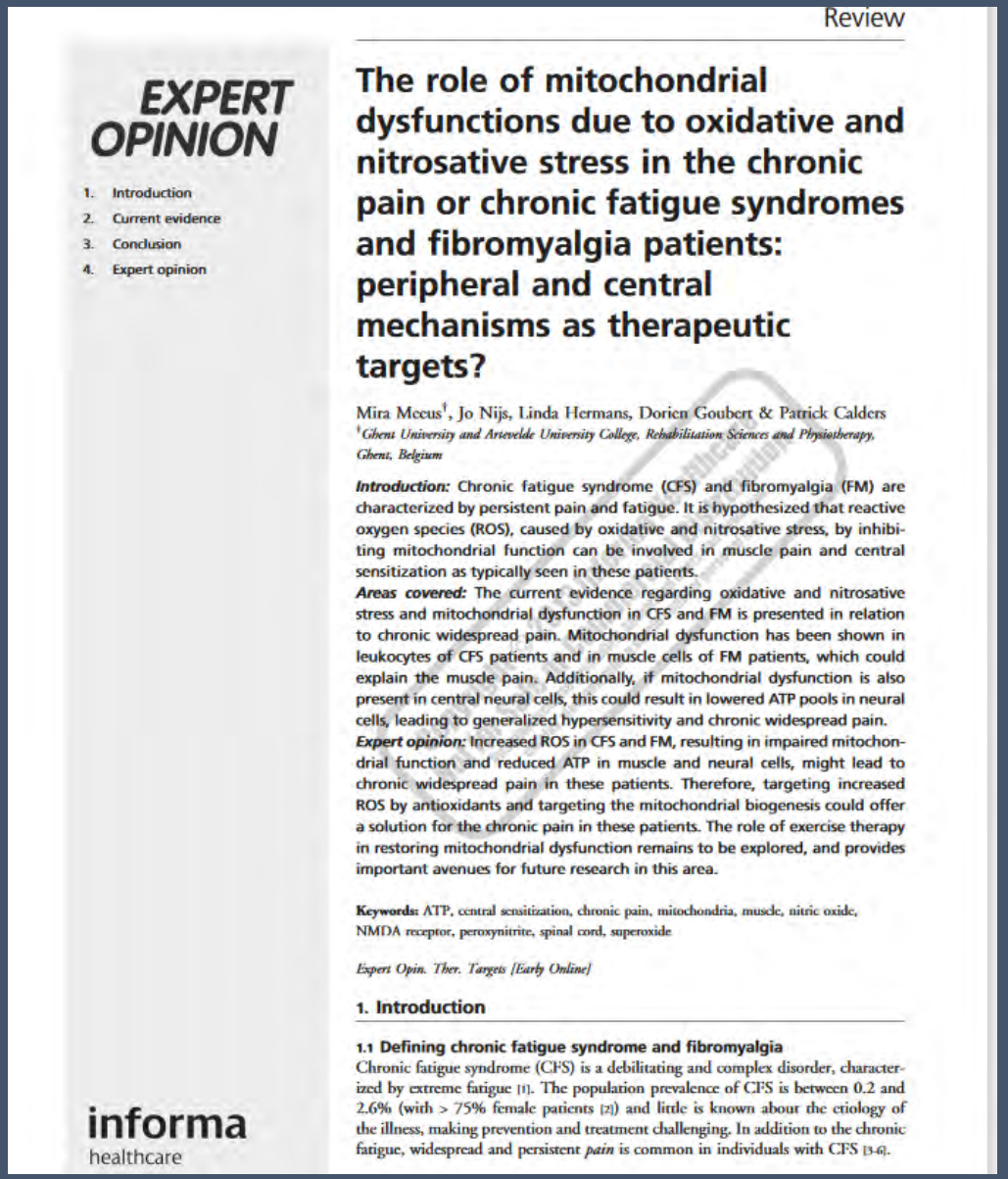
Our results provide evidence for whether there are functional differences in the mitochondrial respiratory capacities of adipocytes from lean and obese subjects and between large and small adipocytes.

MATERIALS AND METHODS

Subjects. Subcutaneous adipose tissue was obtained from female patients who underwent elective abdominal surgery (Table 1). All subjects had no evidence of metabolic or infectious diseases and did not take any medication. Each subject provided written, informed

Address for reprint requests and other correspondence: T. Skurk, Else Kröner-Fresenius-Center for Nutritional Medicine (EKFZ), Technische Universität München, Gregor-Mendel-Str. 2, Freising, Germany (e-mail: skurk@tum.de).

Lowered ATP levels lead to hypersensitivity and chronic widespread pain



Review

EXPERT OPINION

1. Introduction
2. Current evidence
3. Conclusion
4. Expert opinion

The role of mitochondrial dysfunctions due to oxidative and nitrosative stress in the chronic pain or chronic fatigue syndromes and fibromyalgia patients: peripheral and central mechanisms as therapeutic targets?

Mira Meeus[†], Jo Nijs, Linda Hermans, Dorien Goubert & Patrick Calders
[†]*Ghent University and Artevelde University College, Rehabilitation Sciences and Physiotherapy, Ghent, Belgium*

Introduction: Chronic fatigue syndrome (CFS) and fibromyalgia (FM) are characterized by persistent pain and fatigue. It is hypothesized that reactive oxygen species (ROS), caused by oxidative and nitrosative stress, by inhibiting mitochondrial function can be involved in muscle pain and central sensitization as typically seen in these patients.
Areas covered: The current evidence regarding oxidative and nitrosative stress and mitochondrial dysfunction in CFS and FM is presented in relation to chronic widespread pain. Mitochondrial dysfunction has been shown in leukocytes of CFS patients and in muscle cells of FM patients, which could explain the muscle pain. Additionally, if mitochondrial dysfunction is also present in central neural cells, this could result in lowered ATP pools in neural cells, leading to generalized hypersensitivity and chronic widespread pain.
Expert opinion: Increased ROS in CFS and FM, resulting in impaired mitochondrial function and reduced ATP in muscle and neural cells, might lead to chronic widespread pain in these patients. Therefore, targeting increased ROS by antioxidants and targeting the mitochondrial biogenesis could offer a solution for the chronic pain in these patients. The role of exercise therapy in restoring mitochondrial dysfunction remains to be explored, and provides important avenues for future research in this area.

Keywords: ATP, central sensitization, chronic pain, mitochondria, muscle, nitric oxide, NMDA receptor, peroxynitrite, spinal cord, superoxide

Expert Opin. Ther. Targets [Early Online]

1. Introduction

1.1 Defining chronic fatigue syndrome and fibromyalgia

Chronic fatigue syndrome (CFS) is a debilitating and complex disorder, characterized by extreme fatigue [1]. The population prevalence of CFS is between 0.2 and 2.6% (with > 75% female patients [2]) and little is known about the etiology of the illness, making prevention and treatment challenging. In addition to the chronic fatigue, widespread and persistent *pain* is common in individuals with CFS [3-6].

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Mesenchymal Stem Cells Synergize with 635, 532, and 405 nm Laser Wavelengths in Renal Fibrosis: A Pilot Study

Megan O'Connor, MS,[1] Rachana Patil, MS,[1] Jiangzhou Yu, MD, PhD,[1] Richard Hickey, BS,[1] Kavitha Premanand, MS,[1] Andre Kajdacsy-Balla, MD, PhD,[2] Enrico Benedetti, MD,[1,3] and Amelia Bartholomew, MD, MPH, FACS[1]

Abstract

Objective: To address whether a single treatment of one of three visible light wavelengths, 635, 532, and 405 nm (constant wave, energy density 2.9 J/m^2), could affect the hallmarks of established renal fibrosis and whether these wavelengths could facilitate mesenchymal stem cell (MSC) beneficence. ***Background data:*** Chronic kidney disease is a global health problem with only 20% receiving care worldwide. Kidneys with compromised function have ongoing inflammation, including increased oxidative stress and apoptosis, peritubular capillary loss, tubular atrophy, and tubulointerstitial fibrosis. Promising studies have highlighted the significant potential of MSC-based strategies to mitigate fibrosis; however, reversal of established fibrosis has been problematic, suggesting that methods to potentiate MSC effects require further development. Laser treatments at visible wavelengths have been reported to enhance mitochondrial potential and available cellular ATP, facilitate proliferation, and inhibit apoptosis. We hypothesized that laser-delivered energy might provide wavelength-specific effects in the fibrotic kidney and enhance MSC responses. ***Materials and methods:*** Renal fibrosis, established in C57BL6 mice following 21 days of unilateral ureter obstruction (UUO), was treated with one of three wavelengths alone or with autologous MSC. Mitochondrial activity, cell proliferation, apoptosis, and cytokines were measured 24 h later. ***Results:*** Wavelengths 405, 532, and 635 nm all significantly synergized with MSC to enhance mitochondrial activity and reduce apoptosis. Proliferative activity was observed in the renal cortices following combined treatment with the 532 nm laser and MSC; endothelial proliferation increased in response to the 635 nm laser alone and to the combined effects of MSC and the 405 nm wavelength. Reductions of transforming growth factor-β were observed with 532 nm alone and when combined with MSC. ***Conclusions:*** Specific wavelengths of laser energy appear to induce different responses in renal fibrotic tissue. These findings support further study in the development of a customized laser therapy program of combined wavelengths to optimize MSC effects in the treatment of renal fibrosis.

Keywords: kidney fibrosis, laser wavelength, mesenchymal stem cell, tissue regeneration

Background

Chronic kidney disease is considered a worldwide health crisis; only 20% of affected individuals are treated worldwide.[1,2] The financial burden of ongoing treatment remains a significant obstacle to care. Strategies aimed at facilitating permanent endogenous recovery of kidney function may circumvent this obstacle. Kidneys with compromised function have developed structural changes in response to ongoing inflammation, including increased oxidative stress and apoptosis, peritubular capillary loss, tubular atrophy, and tubulointerstitial fibrosis.[3–8] Transforming growth factor-β (TGF-β) has been implicated as a key player in epithelial–mesenchymal transition, a process that contributes pathologically to fibrosis and excessive deposition of extracellular matrix.[8–8]

While mesenchymal stem cell (MSC) have shown to improve acute kidney injury, their effect in chronic fibrotic kidney disease has been less effective.[9] MSC have been reported to suppress some of the underlying inflammatory responses and oxidative stress associated with fibrosis, improve regulation of matrix deposition and remodeling, and inhibit the TGF-β pathway.[9–14] Encouraging findings have shown reduced albuminuria, collagen IV deposition, and loss of peritubular capillaries, but MSC alone could not completely reverse or restore function, despite their abilities to facilitate endothelial and epithelial proliferation.[15,16]

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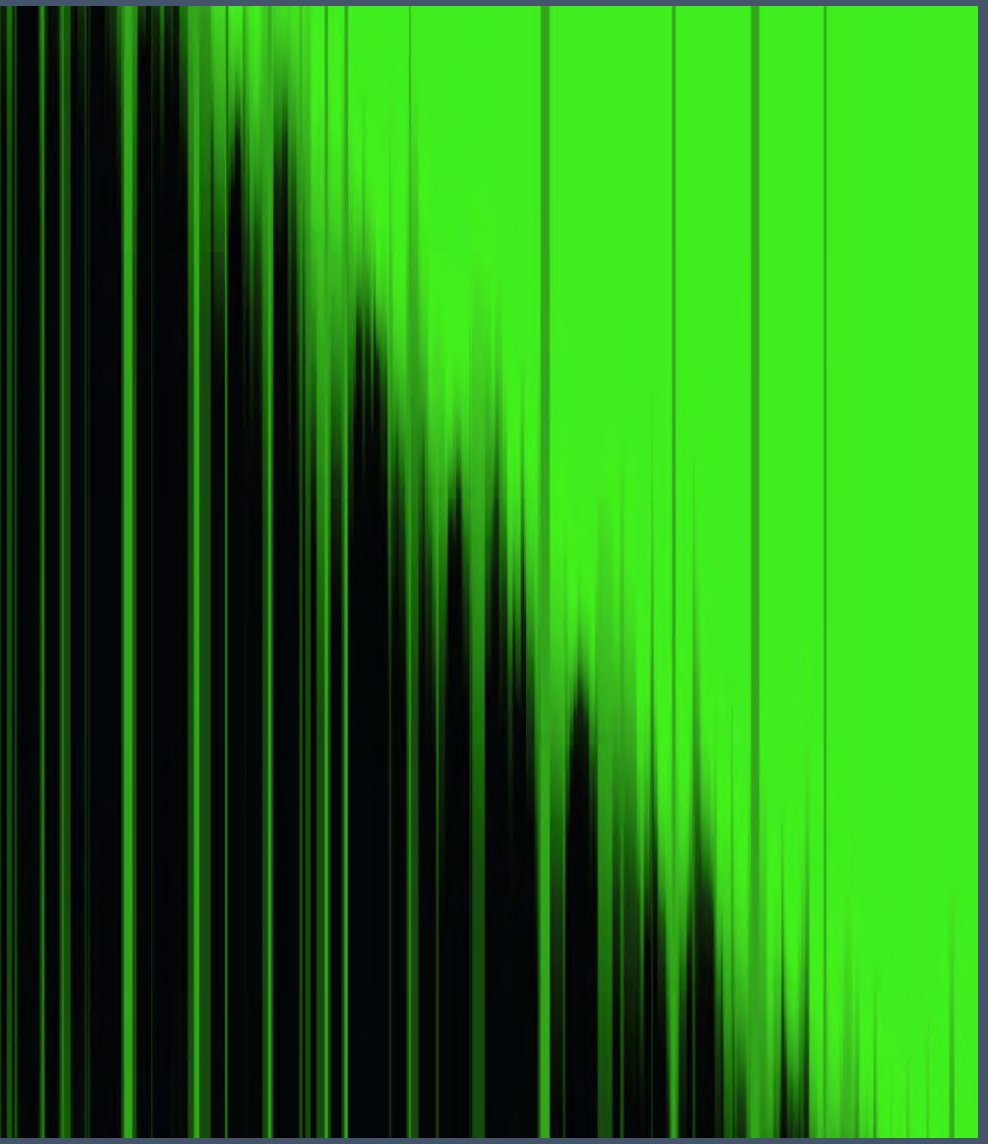
University of Illinois at Chicago

635nm
Mitochondrial Activity
Proliferative activity
Production of IL-10

405nm
Reduction of Apoptotic cells on fibrous tissues
Improved breakdown of scar tissue due to high eV

532nm
Reduction of TGF-B
Biggest stimulation of stem cells



Green Wavelength

Bone tissue healing

- Merigo et al – 532nm green laser has positive effect on osteogenic differentiation of murine bone marrow stromal cells (BMSC)[22]
- Production of BMSC **facilitates bone repair**. BMSCs also facilitate nerve regeneration[23]

ELSEVIER

Journal of Photochemistry and Photobiology B: Biology

Volume 155, February 2016, Pages 130-136

Green laser light irradiation enhances differentiation and matrix mineralization of osteogenic cells ☆, ☆☆

Elisabetta Merigo [a, b, c, d], Sebastien Bouvet-Gerbettaz [a, b, c, d], Florian Boukhechba [a, b, c, e], Jean-Paul Rocca [d], Carlo Fornaini [d], Nathalie Rochet [a, b, c]

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Highlights

- KTP LLLT may enhance the osteogenic differentiation of bone marrow stromal cells.
- KTP LLLT may enhance the mineralization of the extracellular matrix.
- KTP laser may be useful on bone tissue engineering models *in vivo* and *in vitro*.

Abstract

Background and objective

Low level laser therapy (LLLT) in both infrared and visible light is a therapeutic tool ever more proposed in clinical practice in different fields. The effect of near infrared LLLT has been described in a growing number of scientific publications related to bone tissue healing, both *in vitro* and *in vivo*. More recently, green visible light using potassium-titanyl-phosphate KTiOPO4 (KTP, 532 nm) laser has been

- Kassak et al – exposure of 532nm low power green laser led to significant increase in mitochondrial transmembrane potential of 13%
- Changes in mitochondrial transmembrane potential integral to cell life and in normal cell function is essential for ATP synthesis.

Gen. Physiol. Biophys. (2005), **24**, 209—220

Mitochondrial Alterations Induced by 532 nm Laser Irradiation

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[2] *Department of General Biophysics, University of Lodz, Lodz, Poland*

Abstract. Mitochondrial alterations were monitored after low power green laser (532 nm, 30 mW) irradiation in the case of whole cells (B-14) and isolated mitochondria (from Wistar rat heart). 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium-bromide (MTT) assay products were significantly higher (by 8%) in irradiated B-14 cells as compared to non-irradiated controls. Mitochondrial transmembrane potential of B-14 cells, measured by means of a fluorescent probe 3,3'-dihexyl-oxacarbocyanine iodide ($DiOC_6(3)$), significantly increased (by 13%) after exposure to green laser irradiation. Another MTT assay was used for isolated mitochondria suspensions in order to examine the effect of green laser irradiation on stimulation of processes related to oxidative phosphorylation. It revealed 31.3%-increase in MTT assay products in irradiated mitochondria as compared to controls. Laser irradiation of isolated mitochondria suspension did not significantly change 1,6-diphenyl-1,3,5-hexatriene (DPH) fluorescence anisotropy, indicating that mitochondrial membrane fluidity was not affected by laser light. Fluorescence emission spectra of irradiated as well as non-irradiated mitochondria suspensions showed fluorescence maximum at 635 nm, corresponding to emission of Protoporphyrin IX, which was significantly lower (by 20.7%) in irradiated sample.

Key words: Mitochondria — Transmembrane potential — Oxidative phosphorylation — Membrane fluidity — Laser irradiation — Protoporphyrin IX

Introduction

The first publication about low-level laser therapy (LLLT) appeared more than 30 years ago (Kovács et al. 1974). Since then, the effectiveness and applicability of a variety of light sources, in the treatment of a wide range of medical conditions

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420 nm and 540 nm wavelengths more effective in stimulating osteoblast differentiation compared to 660 nm and 810 nm

SCIENTIFIC REPORTS

OPEN

Photobiomodulation (blue and green light) encourages osteoblastic-differentiation of human adipose-derived stem cells: role of intracellular calcium and light-gated ion channels

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Yuguang Wang[1,2,3,4], Ying-Ying Huang[3,4], Yong Wang[1,2], Peijun Lyu[1,2] & Michael R. Hamblin[3,4,5]

Human adipose-derived stem cells (hASCs) have the potential to differentiate into several different cell types including osteoblasts. Photobiomodulation (PBM) or low level laser therapy (LLLT) using red or near-infrared wavelengths has been reported to have effects on both proliferation and osteogenic differentiation of stem cells. We examined the effects of delivering four different wavelengths (420 nm, 540 nm, 660 nm, 810 nm) at the same dose (3 J/cm^2) five times (every two days) on hASCs cultured in osteogenic medium over three weeks. We measured expression of the following transcription factors by RT-PCR: RUNX2, osterix, and the osteoblast protein, osteocalcin. The 420 nm and 540 nm wavelengths were more effective in stimulating osteoblast differentiation compared to 660 nm and 810 nm. Intracellular calcium was higher after 420 nm and 540 nm, and could be inhibited by capsazepine and SKF96365, which also inhibited osteogenic differentiation. We hypothesize that activation of light-gated calcium ion channels by blue and green light could explain our results.

Human adipose-derived stem cells (hASCs) have emerged as a popular and versatile tool in the field of regenerative medicine[1]. Adipose tissue is usually isolated in the form of fat removed during liposuction procedures. This tissue represents an abundant and accessible source of adult stem cells that can be purified from the lipoaspirate, with the ability to differentiate along multiple lineage pathways[2]. hASCs have been shown to be very similar (in terms of markers expressed on their surface and in their differentiation potential) to bone marrow-derived mesenchymal stem cells (BMDMSC)[3].

Many surgical and orthopedic procedures require the reconstruction of significant defects in bone, which are beyond the already excellent capacity of natural bone to heal, because they are too large[4]. Autologous bone graft which is usually harvested from the iliac crest, is considered to be the gold standard material for bone regeneration in orthopedic surgery[5]. However the autologous bone graft procedure has limitations including donor site morbidity, limited amounts, and a requirement for a second surgical procedure.

To overcome these limitations, researchers have proposed the use of HADSC to provide a source of cells that can differentiate and proliferate into osteogenic cells (osteoblasts) under the influence of the appropriate molecular signals[6]. These signals can be partly provided by an appropriate scaffold with the correct properties: a three-dimensional structure, a composition consisting of polymers (e.g. poly-lactic-co-glycolic acid), proteins (e.g. collagen) and minerals (e.g. hydroxyapatite)[7]. In addition to the correct scaffold exogenous growth factors

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- Lubart et al examined effects of red (632 nm), green (540 nm) and infrared (904 nm) light sources on peripheral nervous system[18]
- No significant difference found for the action potential of the nerve with infrared and control group
- Only red and green wavelengths had effect on the **compound action potential (CAP) of the nerve**
- **Green light much more effective than red**

A LIGHT SOURCE FOR PHOTOTHERAPY

R. Lubart*§, S. Rochkind†, U. Sharon‡ and M. Nissan§

*Department of Physics, Bar-Ilan University, Israel, †Department of Neurosurgery, Ichilov Hospital, Israel, ‡Pan Engineering Ltd., Israel, §Technion, Haifa, Israel

The effects of red (632 nm), green (540 nm) and infrared (904 nm) light sources on the peripheral nervous system were examined. Only the red and green wavelengths have an effect on the Compound Action Potential (CAP) of the nerve. This agrees with our hypothesis that porphyrins absorb the irradiated light. A light source which meets the requirements and specifications of our research is being built.

key words: Low power light source (LPS) Peripheral nervous system (PNS) Compound action potential (CAP) Porphyrins

Introduction

Low power lasers and other irradiation sources of low power light are used both clinically and experimentally throughout the world, but there are still no conclusive results as to the beneficial effects of these light sources.[1] The effect of light upon the peripheral nerves has been dealt with by various investigators. Da Ren *et al.*[2] sectioned and sutured back the sciatic nerve in rats and found HeNe laser delivering 150 mJ to the point of suture increased the degeneration of damaged fibres. Walker,[3, 4] reported temporary suppression of clonus in humans and the appearance of laser-induced somatosensory evoked potentials, using 1 mW HeNe pulsed laser delivering 0.5 J/cm^2 transcutaneously.

Wu[5] reported a failure in confirming Walker's report. Also Basford[6] did not find 1 mW HeNe laser to alter distal sensory latencies action potentials. Greathouse and Currier[7] used pulsed I.R. laser (904 nm) and were unable to detect any change in the nerve conduction.

Our group is working on the effects of low power light on the nervous system. We performed most of our previous tests using HeNe laser (632.8 nm) as reported in detail in our previous papers.[8, 9] We found that a 1 mW HeNe laser cannot reach the nerve when irradiated transcutaneously. A more powerful laser is needed to cause the (CAP) of an injured nerve to reach values above the normal pre-crush ones.[8] We also reported on direct irradiation of exposed sciatic nerves in rats.[9] We have recently found[10] that at low radiation doses, singlet oxygen ($^1O_2$) is produced by energy transfer from a photosensitizer, most probably haemotoporphyrin which is known to be present in every cell.

The present work represents results which relate to the effect of different wavelengths on injured nerves. According to these results and those resulting from our understanding of laser–tissue mechanism, we propose a new light source for light therapy.

Materials and Methods

The present study was carried out on 67 rats of the Sprague Dawley kind. The rats were anaesthetized and the sciatic nerve was exposed surgically. CAP was measured from the bare nerve for 10 min using specially designed electrodes in order to establish the normal CAP. The nerve was then crushed and CAP measured for another 10 min, allowing the nerve to reach steady-state post-crush value. Up to this point all rats were treated according to the same protocol. Following the crush, the rats were divided into five groups, A to E. Group A (10 rats) served as control. This group did not receive irradiation or any other treatment following the crush. CAP was recorded for 45 min, during which it reached a stable constant value. In group B, 22 rats, the CAP was recorded for 10 min following the crush, reaching a constant value. The nerve was then irradiated for different intervals of time using a green HeNe laser (0.5 mW 540 nm, Lasotronic Switzerland) and the CAP was recorded again. The optimal time for irradiating with this laser was found to be 30 min (900 mJ). Similar protocol was used in the next two groups C and D, using a 0.5 mW pulsed I.R. laser (904 nm, Lasotronic Switzerland) instead of the green HeNe one. In group C the laser was pulsed at 900 Hz and in group D pulsed at 1500 Hz. In the last group E, a 0.3 mW red HeNe CW laser (632.8 nm, Spectra-Physics) was used. The optimal time for irradiating with the red laser was 7 min (126 mJ).

Addressee for correspondence: Dr R. Lubart, Physics Department, Bar-Ilan University, Ramat-Gan 52100, Israel.

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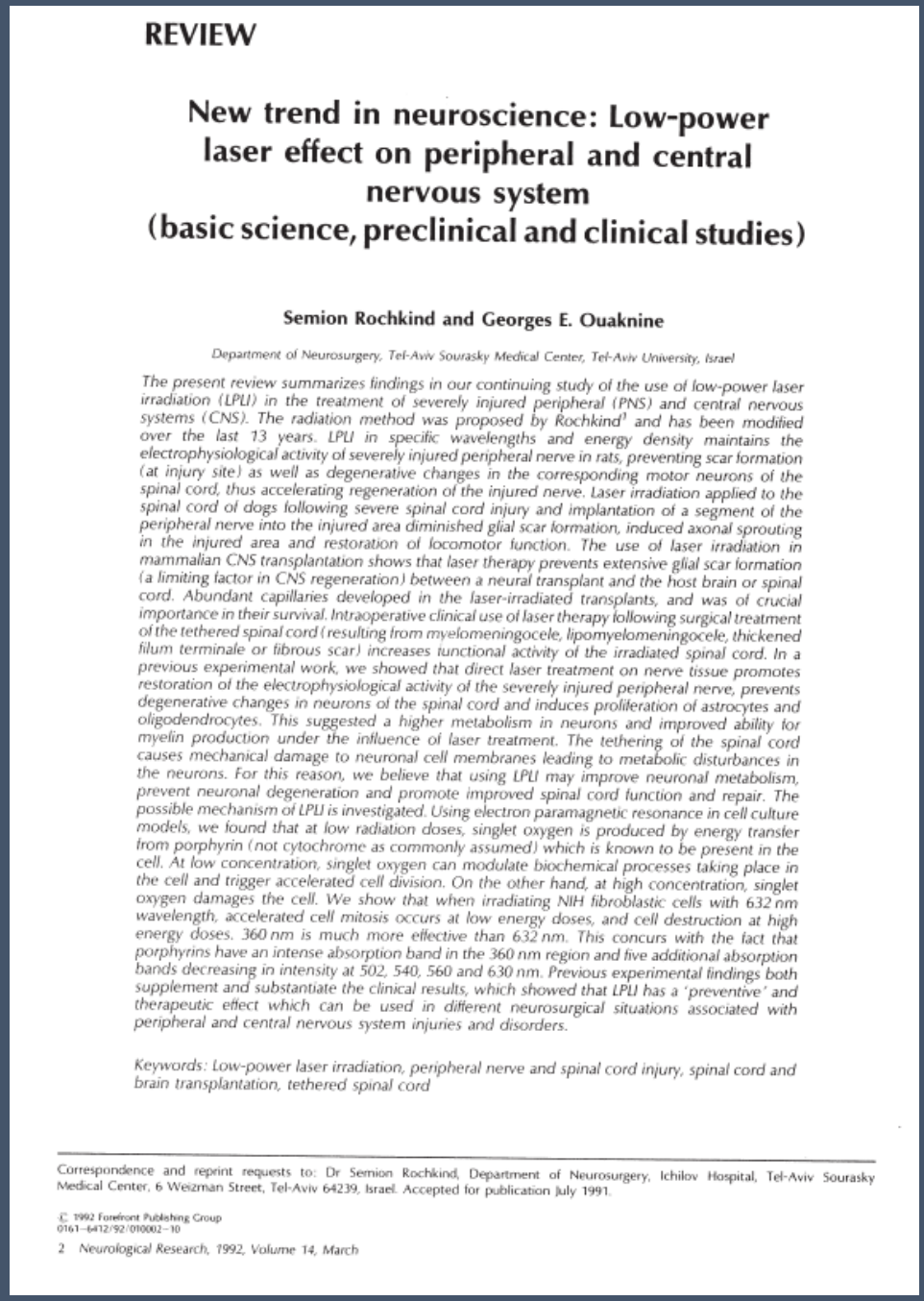
Injured nerves

The continuation of this work with different wavelengths showed **green** 540 nm more effective than 632 nm



REVIEW

New trend in neuroscience: Low-power laser effect on peripheral and central nervous system (basic science, preclinical and clinical studies)

Semion Rochkind and Georges E. Ouaknine

Department of Neurosurgery, Tel-Aviv Sourasky Medical Center, Tel-Aviv University, Israel

The present review summarizes findings in our continuing study of the use of low-power laser irradiation (LPLI) in the treatment of severely injured peripheral (PNS) and central nervous systems (CNS). The radiation method was proposed by Rochkind[1] and has been modified over the last 13 years. LPLI in specific wavelengths and energy density maintains the electrophysiological activity of severely injured peripheral nerve in rats, preventing scar formation (at injury site) as well as degenerative changes in the corresponding motor neurons of the spinal cord, thus accelerating regeneration of the injured nerve. Laser irradiation applied to the spinal cord of dogs following severe spinal cord injury and implantation of a segment of the peripheral nerve into the injured area diminished glial scar formation, induced axonal sprouting in the injured area and restoration of locomotor function. The use of laser irradiation in mammalian CNS transplantation shows that laser therapy prevents extensive glial scar formation (a limiting factor in CNS regeneration) between a neural transplant and the host brain or spinal cord. Abundant capillaries developed in the laser-irradiated transplants, and was of crucial importance in their survival. Intraoperative clinical use of laser therapy following surgical treatment of the tethered spinal cord (resulting from myelomeningocele, lipomyelomeningocele, thickened filum terminale or fibrous scar) increases functional activity of the irradiated spinal cord. In a previous experimental work, we showed that direct laser treatment on nerve tissue promotes restoration of the electrophysiological activity of the severely injured peripheral nerve, prevents degenerative changes in neurons of the spinal cord and induces proliferation of astrocytes and oligodendrocytes. This suggested a higher metabolism in neurons and improved ability for myelin production under the influence of laser treatment. The tethering of the spinal cord causes mechanical damage to neuronal cell membranes leading to metabolic disturbances in the neurons. For this reason, we believe that using LPLI may improve neuronal metabolism, prevent neuronal degeneration and promote improved spinal cord function and repair. The possible mechanism of LPLI is investigated. Using electron paramagnetic resonance in cell culture models, we found that at low radiation doses, singlet oxygen is produced by energy transfer from porphyrin (not cytochrome as commonly assumed) which is known to be present in the cell. At low concentration, singlet oxygen can modulate biochemical processes taking place in the cell and trigger accelerated cell division. On the other hand, at high concentration, singlet oxygen damages the cell. We show that when irradiating NIH fibroblastic cells with 632 nm wavelength, accelerated cell mitosis occurs at low energy doses, and cell destruction at high energy doses. 360 nm is much more effective than 632 nm. This concurs with the fact that porphyrins have an intense absorption band in the 360 nm region and five additional absorption bands decreasing in intensity at 502, 540, 560 and 630 nm. Previous experimental findings both supplement and substantiate the clinical results, which showed that LPLI has a 'preventive' and therapeutic effect which can be used in different neurosurgical situations associated with peripheral and central nervous system injuries and disorders.

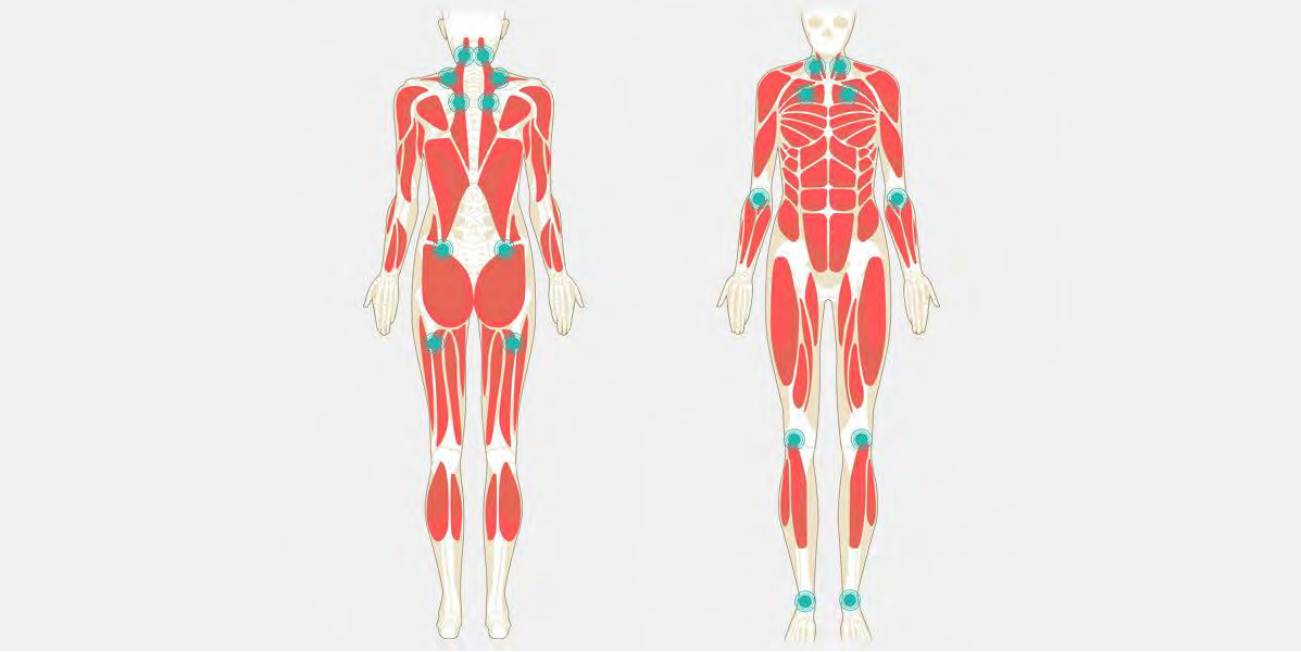
Keywords: Low-power laser irradiation, peripheral nerve and spinal cord injury, spinal cord and brain transplantation, tethered spinal cord

Correspondence and reprint requests to: Dr Semion Rochkind, Department of Neurosurgery, Ichilov Hospital, Tel-Aviv Sourasky Medical Center, 6 Weizman Street, Tel-Aviv 64239, Israel. Accepted for publication July 1991.

0161-6412/92/010002-10

Green light/fibromyalgia

Fibromyalgia patients exposed to green light had significant improvements in their pain, sleep, and quality of life



L Martin, F Porreca, El Mata, et al. **Green Light Exposure Improves Pain and Quality of Life in Fibromyalgia Patients: A Preliminary One-Way Crossover Clinical Trial.** *Pain Medicine*, January 2021;22(1):118–130

Serrage et al. – irradiation with 400nm violet laser increased maximal respiratory rates 53% in Myotubes[29], which are skeletal muscle fibers formed by fusion of myoblasts during developmental stage

Received: 6 November 2018 | Revised: 22 January 2019 | Accepted: 29 January 2019
DOI: 10.1002/jbio.201800411

BIOPHOTONICS

FULL ARTICLE

Differential responses of myoblasts and myotubes to photobiomodulation are associated with mitochondrial number

Hannah J. Serrage[1,2,3*] | Sophie Joanisse[2] | Paul R. Cooper[1] | William Palin[1] | Mohammed Hadis[1] | Owen Darch[3] | Andrew Philp[2,4] | Mike R. Milward[1]

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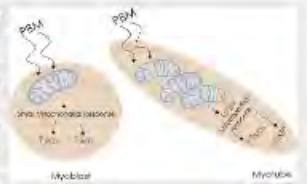
Funding information
Engineering and Physical Sciences Research Council

Objective: Photobiomodulation (PBM) is the application of light to promote tissue healing. Current indications suggest PBM induces its beneficial effects in vivo through upregulation of mitochondrial activity. However, how mitochondrial content influences such PBM responses have yet to be evaluated. Hence, the current study assessed the biological response of cells to PBM with varying mitochondrial contents.



Methods: DNA was isolated from myoblasts and myotubes (differentiated myoblasts), and mitochondrial DNA (mtDNA) was amplified and quantified using a microplate assay. Cells were seeded in 96-wellplates, incubated overnight and subsequently irradiated using a light-emitting diode array (400, 450, 525, 660, 740, 810, 830 and white light, 24 mW/cm^2, 30-240 seconds, 0.72-5.76J/cm^2). The effects of PBM on markers of mitochondrial activity including reactive-oxygen-species and real-time mitochondrial respiration (Seahorse XFe96) assays were assessed 8 hours post-irradiation. Datasets were analysed using general linear model followed by one-way analysis of variance (and post hoc-Tukey tests); $P = 0.05$).

Results: Myotubes exhibited mtDNA levels 86% greater than myoblasts ($P < 0.001$). Irradiation of myotubes at 400, 450 or 810 nm induced 53%, 29% and 47% increases (relative to non-irradiated control) in maximal respiratory rates, respectively ($P < 0.001$). Conversely, irradiation of myoblasts at 400 or 450 nm had no significant effect on maximal respiratory rates.

Conclusion: This study suggests that mitochondrial content may influence cellular responses to PBM and as such explain the variability of PBM responses seen in the literature.

KEYWORDS
low-level laser therapy, low-level light therapy, mitochondria, myogenesis, photobiomodulation

Why violet light

- Violet – enhances physiological outcomes because it has more energy per photon (not power as in MW watts)
- Works in shorter period of time
- More anti-viral, bacterial, fungus
- Has greater response to immune function

Characterizing the antimicrobial properties of 405 nm light

Conclusion:

Violet visible light shown to elicit strong anti-microbicidal effects toward many pathogens

Shehatou C, Logunov SL, Dunman PM, et al. **Characterizing the Antimicrobial Properties of 405 nm Light and the Corning® Light-Diffusing Fiber Delivery System**. *Lasers Surg Med*. 2019;51(10):887-896

Antibacterial/fungal effect of 405 nm

- Irradiation with 405 nm laser has significant bactericidal/fungicidal effect:
  - P. gingivalis – 60% inhibition
  - P. intermedia – 80%

  } 5 mins.

  - C. albicans – 90%

  } 10 mins.
- 405 nm irradiation – strategy for prevention/treatment of endodontic infections

Takahiro I, Seiko T, Yusuke T, et al. *Int J of Photoenergy* 2014

405 Nm light for inactivation of pathogens and its potential role for environmental disinfection and infection control

Conclusion:

- Violet light, particularly 405 Nm light – significant antimicrobial properties against wide range of bacterial and fungal pathogens
- Trial results – 405 NM light system can provide continuous disinfection of air and exposed surfaces in occupied areas of hospital – substantially enhancing cleaning and infection control procedures

M Maclean, K McKenzie, JG Anderson, et al. *J Hosp Infect*, Sept 2014;88(1):1-11

405 Nm in increasing level of nitric oxide (NO) and its effect on viruses

- Stimulates complex 1 of mitochondrial respiratory chain
- Strong anti-bacterial effects by destroying micro-organisms in blood
- Release NO from NO-Hb of micro-circulation
- Can be used as photodynamic therapy (for bacterial, viral, parasitic organisms)

Razzaghi MD, Kamani E. (2020) **Role Low-Power Blue Laser With a Wavelength of 405 Nm in Increasing the Level of Nitric Oxide in Increasing the Resistance of Cells to the Virus (COVID-19) and its Effect on Virus (COVID-19) Mortality in Vitro**. *OSP J Case Rep 2*. JCR-2-118


DEMO TIME

Rebalance

the nervous system

As simple as 1, 2, 3

- Point and shoot – static positioning of the patient and laser
- passive – doctor moves patient and moves laser
- Active – patient moves limb and doctor moves laser

Bonus

Laser cerebellum for brain up-regulation (laser “locomotor lock-in”)

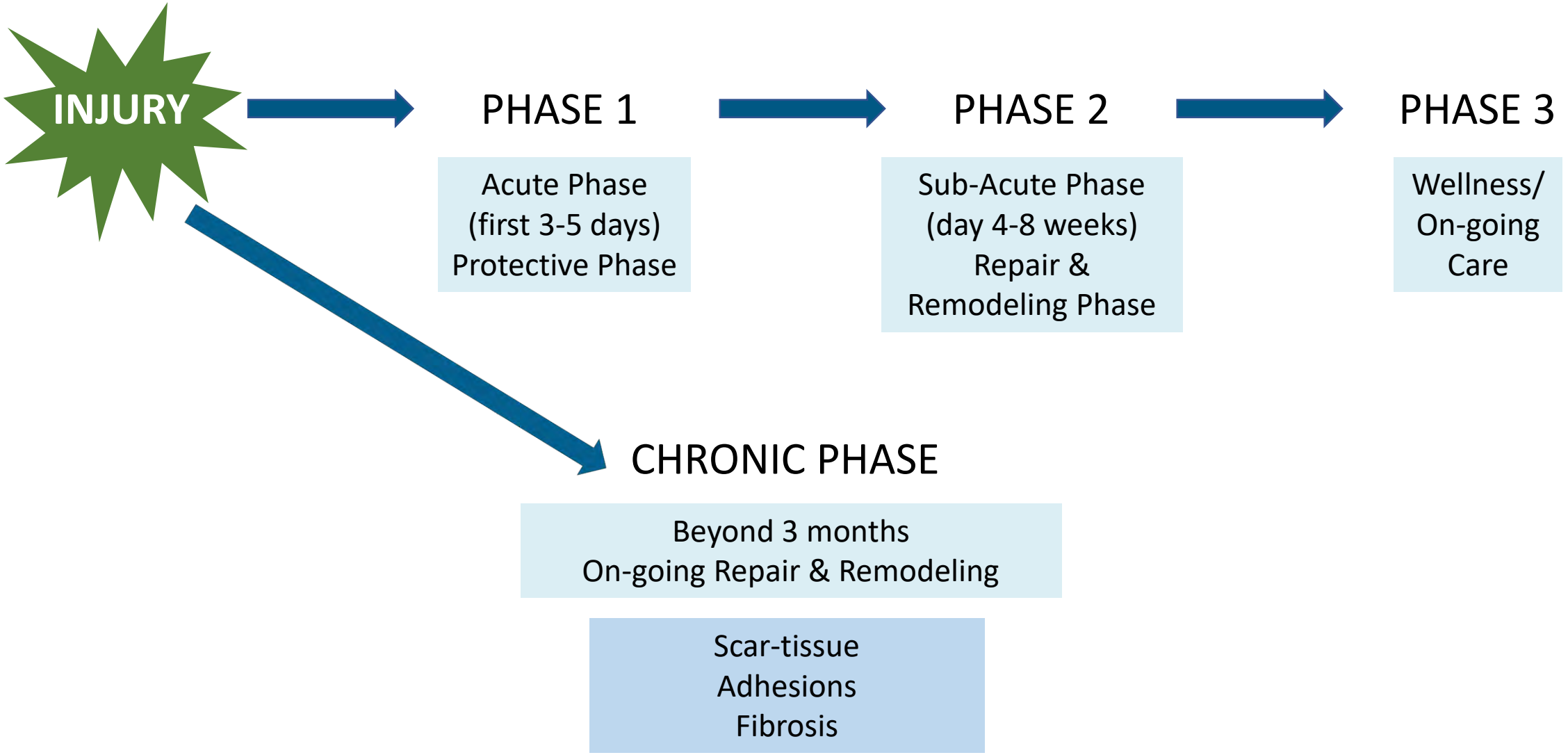
Laser “Locomotor Lock-In”

- Resets NMS in 3D motion
- 4, 9, 33, 60
- Facilitated bodies global integration
- 5 sec. eyes open, 5 sec. eyes closed
- Cross-crawl: right arm – left leg, left arm – right leg
- Aim posterior midline-spine
- Repeat pointing at brain

1) Violet 405 Nm – directly over spinal cord
2) Red 635 Nm – over adjacent nerve roots
3) Both lights - cerebellum

Proprioception after treatment

3 Phases of Care



Dr. Rob's Nutritional Take

- Specific nutrients and oxygen required to sustain heavily used muscle
- Overuse soft tissue injuries result when supply of nutrients are unable to match demands of muscle/tendon region
- Healthy nutrient supply through diet and supplementation assists body with natural function and repair processes

3 Phases of Care

Each individual has a different tolerance threshold and need for variability

EVRL

GVL

FREQUENCY SETTINGS

How to take your patients through acute, sub-acute healing into wellness and performance:

PROTOCOL	LASER A (1)	LASER A (2)	LASER B (1)	LASER B (2)	TIME
1st phase - Acute, for the first 5 days					
Acute1	4	8	25	42	5
Acute2	4	9	33	60	5
Acute3	9	16	42	53	5
2nd phase- sub-acute (repair/remodeling), day 5 through week 8					
Sub-Acute1	8	25	42	48	5
Sub-Acute2	12	30	45	64	5
Sub-Acute3	16	35	48	90	5
3rd phase is wellness and performance.					
Well/Perf1	10	10	10	10	5
Well/Perf2	1	4	9	32	5
Well/Perf3	6	16	26	42	5
Additional Settings					
Chronic	4	40	400	400	5
Nerve Root	4	9	33	60	5
Brain	1	1	1	1	2
Gut	4	4	9	26	2

Elite 8. *My most-used frequencies*

Nerve	4,9,33,60
Muscle	9,16,42,53
Acute injury	4,8,25,42
Chronic scar tissue	8,25,42,279
Brain injury	1,1,1,1
Vagus nerve	10,10,10,10
Gout	6,9,17,40
Infection	5,20,48,625
Brain Neurodegeneration	1,10,(20),40,60

Common muscular-skeletal settings – add to presets

- Ligament: 5-9-125-2720
- Cartilage: 20-45-304-887
- Bursitis: 9-16-142-656
- Edema: 21-33-43-48
- Inflammation: 9-16-42-2720
- Tendonitis: 1-21-25-45
- Neurogenic inflammation: 9-16-33-36
- Wounds: 3-16-24-111
- Peripheral nerve: 2-2-2-2
- Autism: 1-10-5-20

Scar

- EVRL: 8,25,42,279
- Simultaneously with:
  - Myofascial release – directional
  - Instrument-assisted soft-tissue mobilization
  - Percussor

Base Station

Desk top unit – 3 handheld independent laser devices - (1) EVRL and (2) XLR8 lasers, wireless charging station and ability to program all lasers individually using easy-to-use touchscreen GUI's

Configuration:

- 2-Handheld Devices with Dual 640nm/7.5mW Output Laser Diodes
- 1-Handheld Device with One 640nm/7.5mW Laser Diode and One 405nm/<5mW Laser Diode
- Wavelength: 635nm/405nm
- Modulation: Constant Wave (CW) & Variable Hz
- Up to 100 Programmable Memory Channels
- Handheld Device Power Source: 3.7 VDC Rechargeable Lithium-ion Polymer Battery
- Weight: Base Unit with Handheld Laser Devices 2.35 lbs. (1.07kgs.)
- Handheld Lasers less 1lbs. (.30 kgs.) Each
- Laser Class: 2 Device Class II (USA); 2a (EU)

EVRL


ERCHONIA
User Protocols
Preset Protocols
Settings
Quick Start

Effects of a single treatment with two nonthermal laser wavelengths on chronic neck and shoulder pain

- 635 nm + 405 nm synergy
- Patients had pain over 30 days
- 1 treatment for 13 minutes
- VAS – 3 min, 24 & 48 hours later
- Predefined 30% decrease for 65% of subjects

Results:

- 75% achieved ≥30% decrease in VAS pain
- Score remained at 24 and 48 hours

RG Silverman, A Comey, T Sammons. *Medical Devices: Evidence and Research*. 30 August 2019;12:319-25

Effects of a single treatment with two nonthermal laser wavelengths on chronic neck and shoulder pain (cont'd)

Secondary efficacy:

- Change in ROM
- Patient satisfaction improved

Conclusion: Overall, treatment with the red and violet laser out-performed the FDA-approved red laser with respect to change in pain scores and increased shoulder ROM

RG Silverman, A Comey, T Sammons. *Medical Devices: Evidence and Research*. 30 August 2019;12:319-25

Key Concept

To be a Laser Jedi Master, and impact Complex 1, 2, 3, and 4 of the Electron Transport Chain to support mitochondrial health, you will need 3 true, collimated LASER wavelengths:

Violet for Complex 1 and 2,

Green for Complex 3

Red for Complex 4.

My ideal base station

Options:

1) GVL, EVRL, XLR8
2) EVRL, EVRL, GVL
3) GVL, GVL, EVRL

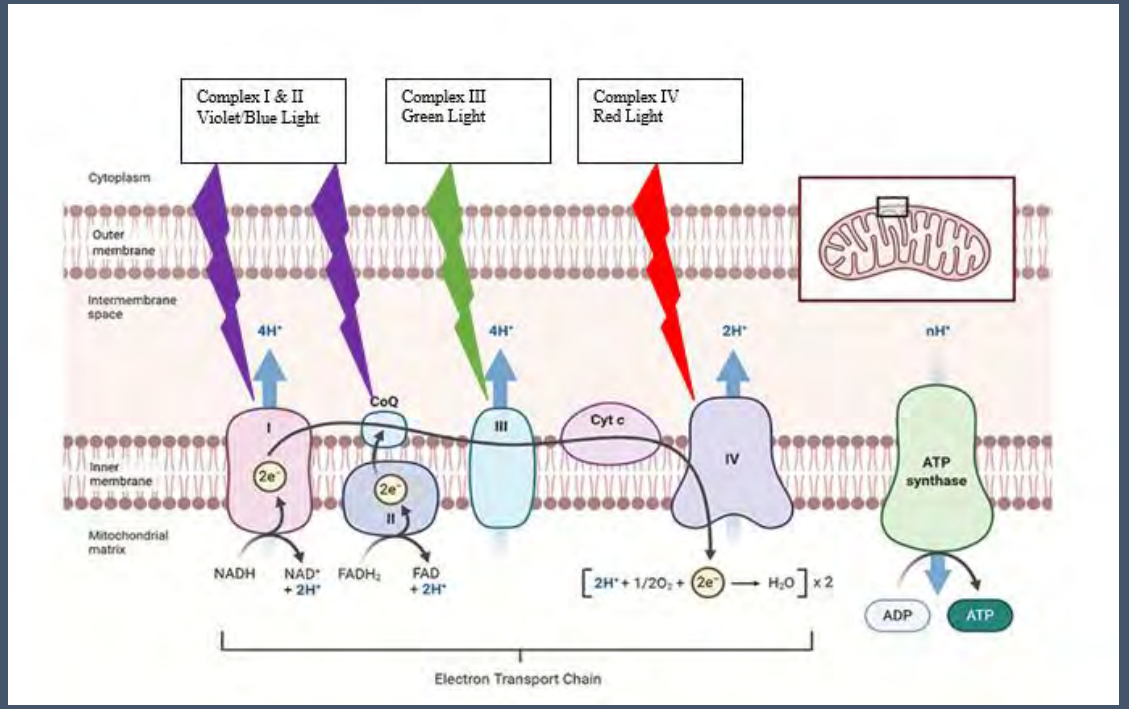
Laser rental

Telemedicine and home care on the rise. Laser rental program should be an integral part of every practice

Rental

Fixing the ETC

- GVL is used in office to upregulate initial ETC complex's (1-3)
- Then the doctor rents out the XLR8 (more affordable device) so the patient can upregulate complex 4 at home



Remote treatment of PD with PBM

Remote application of PBM shown to be effective treatment for a number of clinical signs of PD, with some being maintained for 45 weeks, despite lockdown restrictions

Liebert A, Bicknell B, Laakso EL, et al. **Remote Photobiomodulation Treatment for the Clinical Signs of Parkinson's Disease: A Case Series Conducted During COVID-19**. *Photobiomodul Photomed Laser Surg*. 2022 Feb;40(2):112-122

LLT ORIGINAL ARTICLES

THE PHOTOBIOLOGICAL BASIS OF LOW LEVEL LASER RADIATION THERAPY

Kendric C. Smith

Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305-5105, U.S.A.

Low level laser radiation therapy is effective in a number of clinical situations (e.g. pain relief, wound healing, sports medicine), but the photobiological basis of this therapy is not well-understood. Since both visible and infrared radiations have been shown to be beneficial in such therapies, and since these two radiations differ dramatically in their photochemical and photophysical properties, how can they produce similar results clinically? I propose a modification of the model of Karu[1] to explain this. In her model, visible light produces photochemical changes in photoreceptors in the mitochondria, which alter metabolism, which leads to signal transduction to other parts of the cell (including membranes), which finally leads to the photoresponse (i.e. biostimulation). While visible light probably starts the cascade of metabolic events at the level of the respiratory chain of the mitochondria through photochemical events (probably the photoactivation of enzymes), I propose that because of the photochemical and photophysical properties of infrared radiation, infrared radiation starts the cascade of metabolic events by photophysical effects on the membranes (probably the Ca^{++} channels). Action spectra are needed to quantitate the relative effectiveness of the different wavelengths of radiation, since this can help to identify the photoreceptors for the photobiological response, and to establish the optimum conditions (i.e. wavelength, dose, and treatment schedule) for a particular therapy.

KEY WORDS Photobiological basis of LLLT Action spectra Quantum yield Absorption spectra First law of photochemistry True photochemical sensitivity Model to explain LLLT Photoactivation of enzymes Photomodulation of membranes Ca^{++} channels

Introduction

Low level laser radiation therapy is effective in a number of clinical situations (see below), but the photobiological basis of this therapy is not well understood. Since wavelengths both in the visible region (380–700 nm) and the infrared region (700 nm–1000 μm) of the electromagnetic spectrum are effective in such therapies, and since the radiation in these two wavelength regions differ so dramatically in their photochemical and photophysical properties, how can they produce similar clinical results? This paper proposes a model to answer this question.

Visible Radiation

In the visible region, when a photon is absorbed by a molecule, the electrons of that molecule are raised to a higher energy state. This excited molecule must then lose its extra energy, and it can do so either by re-emitting a photon of longer wavelength (i.e. less energy), as in fluorescence or phosphorescence, or it can lose energy by giving off heat, or it can lose energy by undergoing photochemistry. Photobiological responses are the result of photochemical and/or photophysical changes produced by the absorption of nonionizing electromagnetic radiation (see reference 2).

To gain a better understanding of the photobiological basis of low level laser radiation therapy, laser radiation therapists will have to become better photobiologists. Unfortunately, almost all photobiologists have been self taught, including me, and some of us have been both better teachers and better pupils than others. Even people who call themselves photobiologists sometimes make mistakes about the properties of light. One of these misconceptions is that visible light is 'safe'. The safety of light is not an intrinsic property of the light, rather, it is whether or not the light is absorbed that determines whether the light is safe or not (see reference 2).

The *first law of photochemistry* states that light must be absorbed before photochemistry can occur. This is a very simple but powerful law. If people would just remember this law, many mistakes about the properties of light (or more generally, of nonionizing radiation) would be avoided. The meaning of this law is that if radiation of a particular wavelength is *not absorbed by a system*, then it is *safe for that system*, since no photochemical or photophysical changes can occur. Visible light can be safe for one biological system, and be very

0898-5901/91/010019-06$05.00

"In principle, one photon can activate one enzyme molecule, which in turn can process many thousands of substrate molecules. If the effect of one photon can be amplified biologically, then one does not need a lot of photons to produce an effect.

One just needs to find the proper wavelength of light to stimulate the proper enzyme, which in turn will stimulate the beneficial therapeutic effect."

Kenneth C. Smith, Stanford University School of Medicine

Join

Dr. Rob's Mastermind Laser Group

on FB

FX635/405 – Frequency settings

How to take your patients through acute, sub-acute healing into wellness and performance:

PROTOCOL	Left Hz	Centre Hz	Centre Hz	Right Hz	TIME
1st phase - Acute, for the first 5 days					
Acute1	4, 8	4, 8	8, 25	25, 42	10
Acute2	4, 9	4, 9	9, 33	33, 60	10
Acute3	9, 16	9, 16	16, 42	42, 53	10
2nd phase- sub-acute (repair/remodeling), day 5 through week 8					
Sub-Acute1	8, 25	8, 25	25, 42	42, 48	10
Sub-Acute2	12, 30	12, 30	30, 45	45, 64	10
Sub-Acute3	16, 35	16, 35	35, 48	48, 90	10
3rd phase is wellness and performance					
Well/Perf1	10, 10	10, 10	10, 10	10, 10	10
Well/Perf2	1, 4	1, 4	4, 9	9, 32	10
Well/Perf3	6, 16	6, 16	16, 26	26, 42	10
Additional Settings					
Chronic	4, 10	4, 10	40, 400	400, 400	10
Nerve Root	4, 9	33, 60	4, 9	33, 60	5
Brain	1, 1	20, 40	1, 10	1, 1	10
Gut	4, 4	4, 4	4, 26	9, 26	10

The chief function of the body is to carry the brain around.

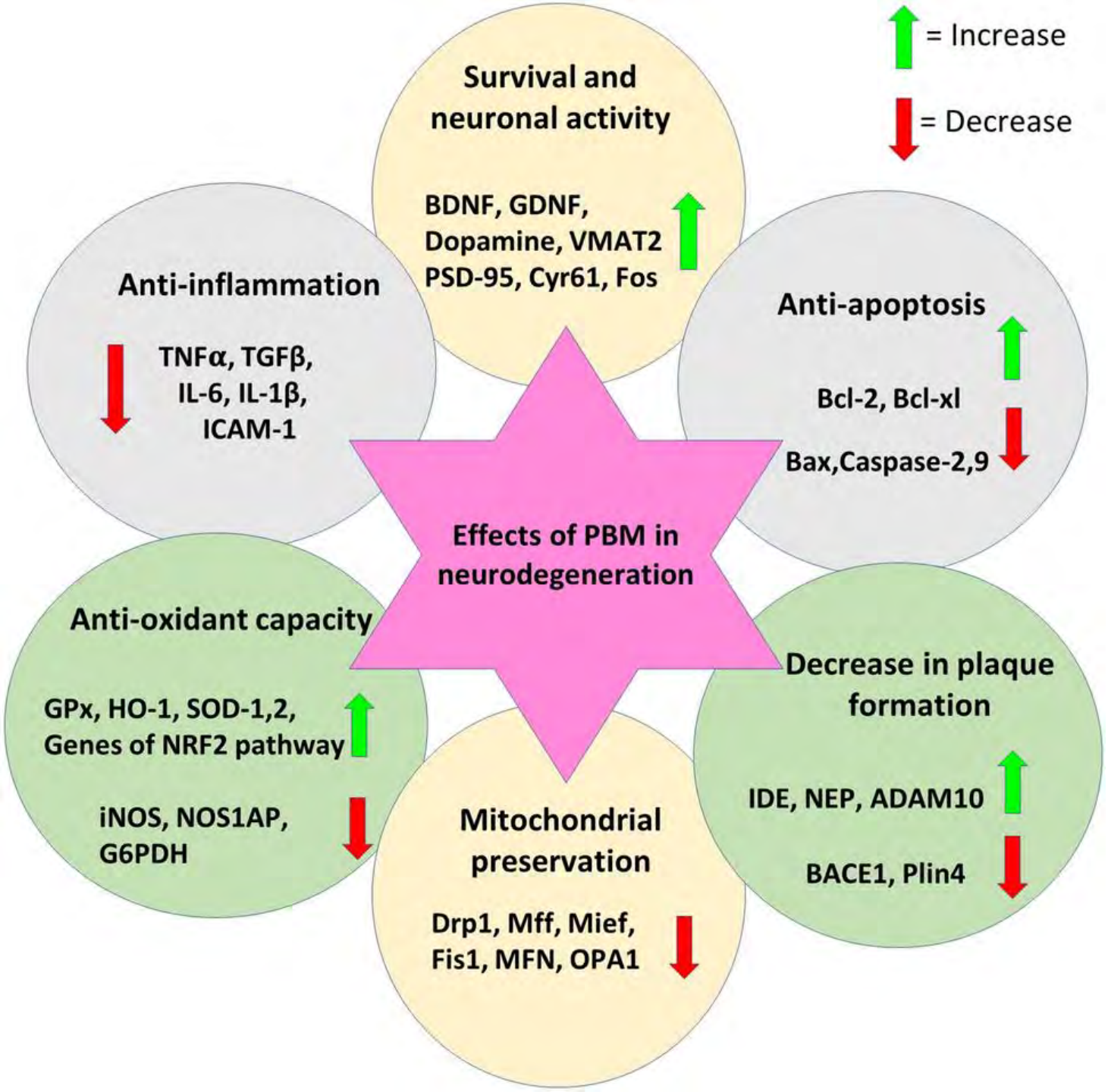
-Thomas A. Edison

Brain facts – don’t use hot laser!

- 2% of human body mass
- 25% of body’s total glucose utilization
- 20% of O_2 consumption
- Most cerebral processes are sensitive to temperature fluctuations
- Temperature fluctuations intrinsically modulate behavioral changes and reflexively generate autonomic responses
- Hypothermia shown to protect against excitotoxicity

Wang H, Wang B, Normoyle KP, et al. **Brain temperature and its fundamental properties: a review for clinical neuroscientists**. *Front Neurosci*. 2014 Oct 8;8:307

Overall effects of PBM on genes and proteins playing different roles in neurodegeneration

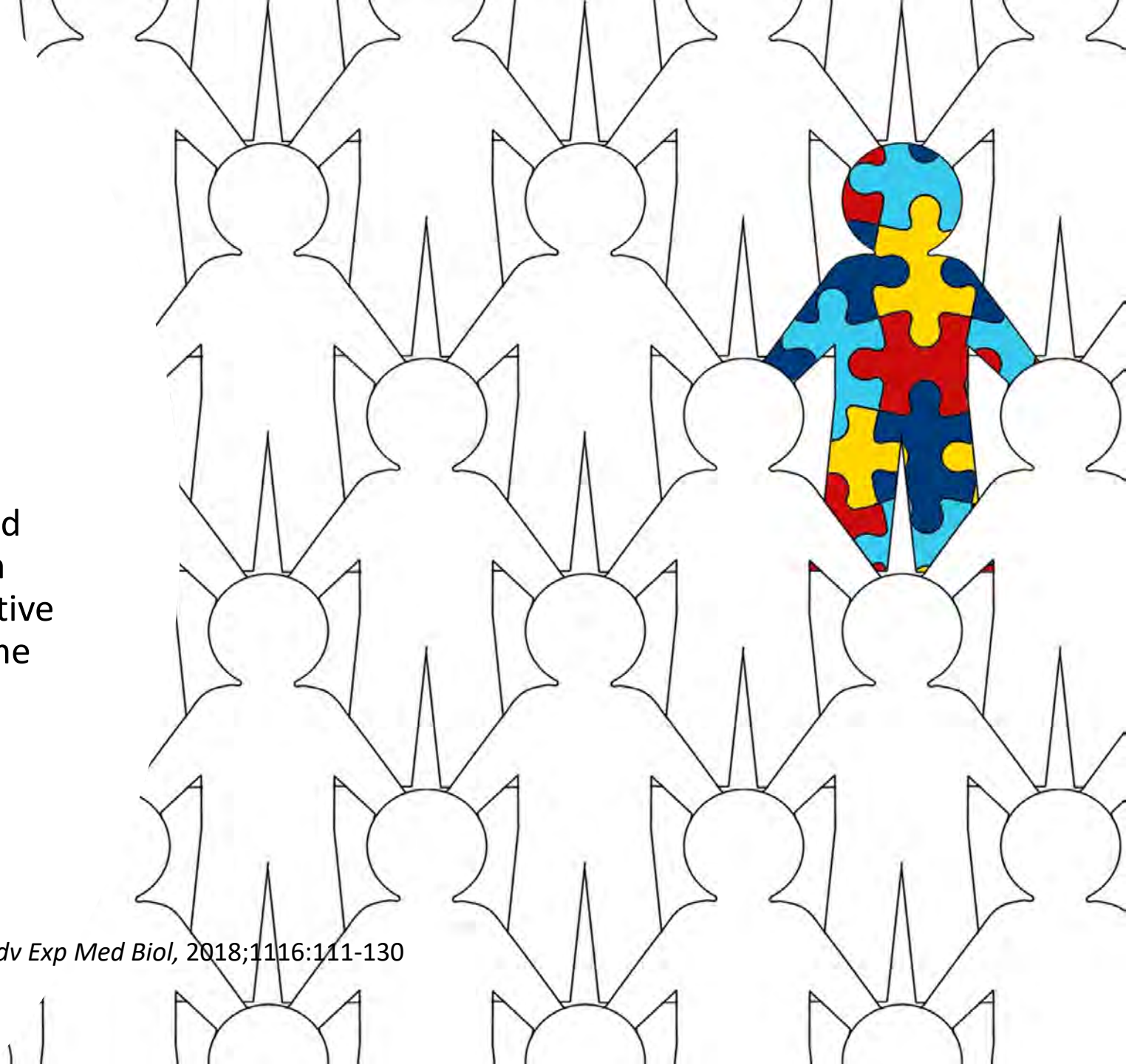


M Bathini, CR Raghushaker, KK Mahato. **The Molecular Mechanisms of Action of Photobiomodulation Against Neurodegenerative Diseases: A Systematic Review**. *Cell Mol Neurobiol* (2020)

Effects of LLLT in autism spectrum disorder

Conclusion:

Study found LLT could be effective tool for reducing irritability and other symptoms and behaviors associated with autistic spectrum disorder in children/adolescence, with positive changes maintained an augmented over time



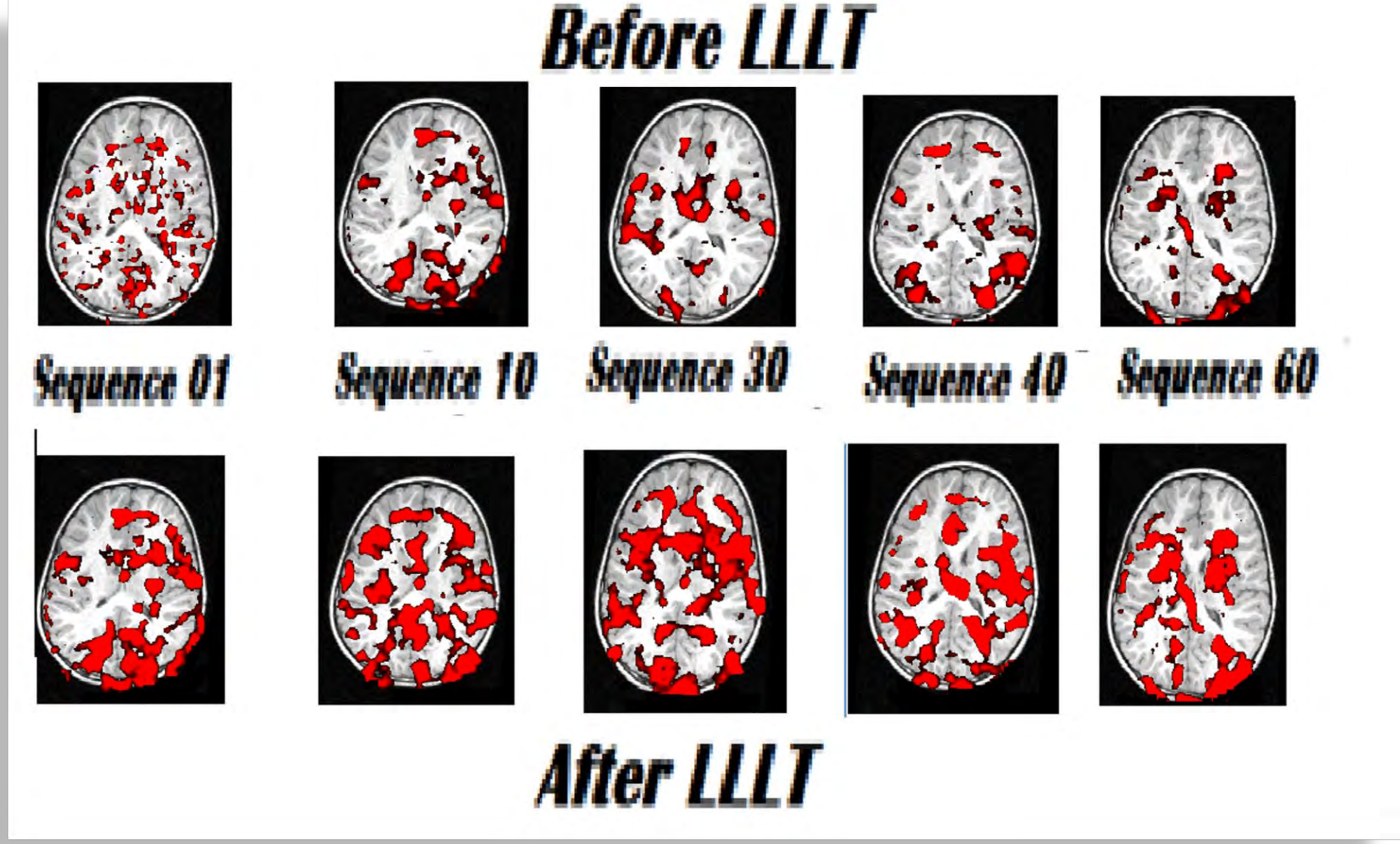
Leisman G, Machado C, Machado Y, Chinchilla-Acosta M. *Adv Exp Med Biol,* 2018;1116:111-130

MRI – fMRI

Autistic Children

Functional magnetic resonance imaging or fMRI measures brain activity by detecting changes associated with blood flow.

This technique relies on the fact that cerebral blood flow and neuronal activation are coupled. When an area of the brain is in use, blood flow to that region also increases.



Follow up assessment of autistic children 6 months after LLLT

- 40 patients
- 21 active, 19 placebo
- 5-minute treatment to base of skull and temporal area, 2 x 4 weeks
- Aberrant Behavior Checklist (ABC) – irritability subscale score:
  - Test vs. placebo – 15.17 in favor of test
  - Improvement in symptoms continued 6 months after assessment

Conclusion:

Study suggests LLLT progressively rearranges neural networks related to complex systems in autism

C Machado, Y Machado, M Chincilla, Y Machado. *The Internet Journal of Neurology*, 2019;21(1)

Follow-up assessment of autistic children 12 months after finishing LLLT

Results:

- 12-month follow-up after completion of LLLT
- Improvement in symptoms continued in patients in test group

Conclusion:

Reaffirmed that clinical improvement maybe patho-physiologically explained because LLLT progressively rearranges anatomical functional and effective connectivity, modifying those neural networks related to complex systems found in autism

C Machado, Y Machado, M Chincilla, Y Machado. *The Internet Journal of Neurology*, 2019;21(2)

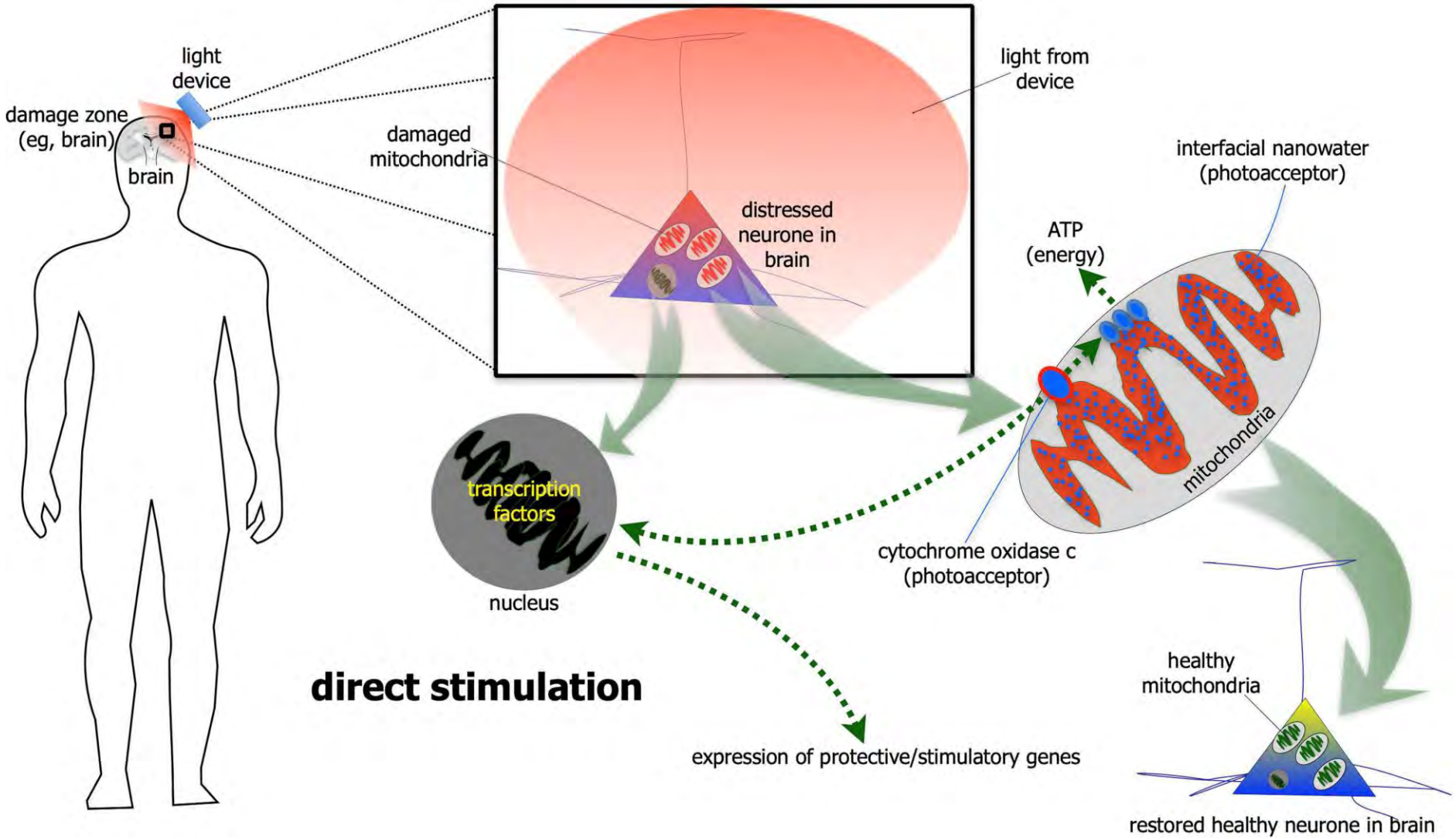
Transcranial LLLT for cognitive function in the elderly

Results: Only older adults who received real PBM exhibited significant improvements in their action selection, inhibition ability, and mental flexibility after vs. mental flexibility after vs. before PBM

Conclusion: PBM may enhance frontal brain functions of older adults in safe and cost-effective manner

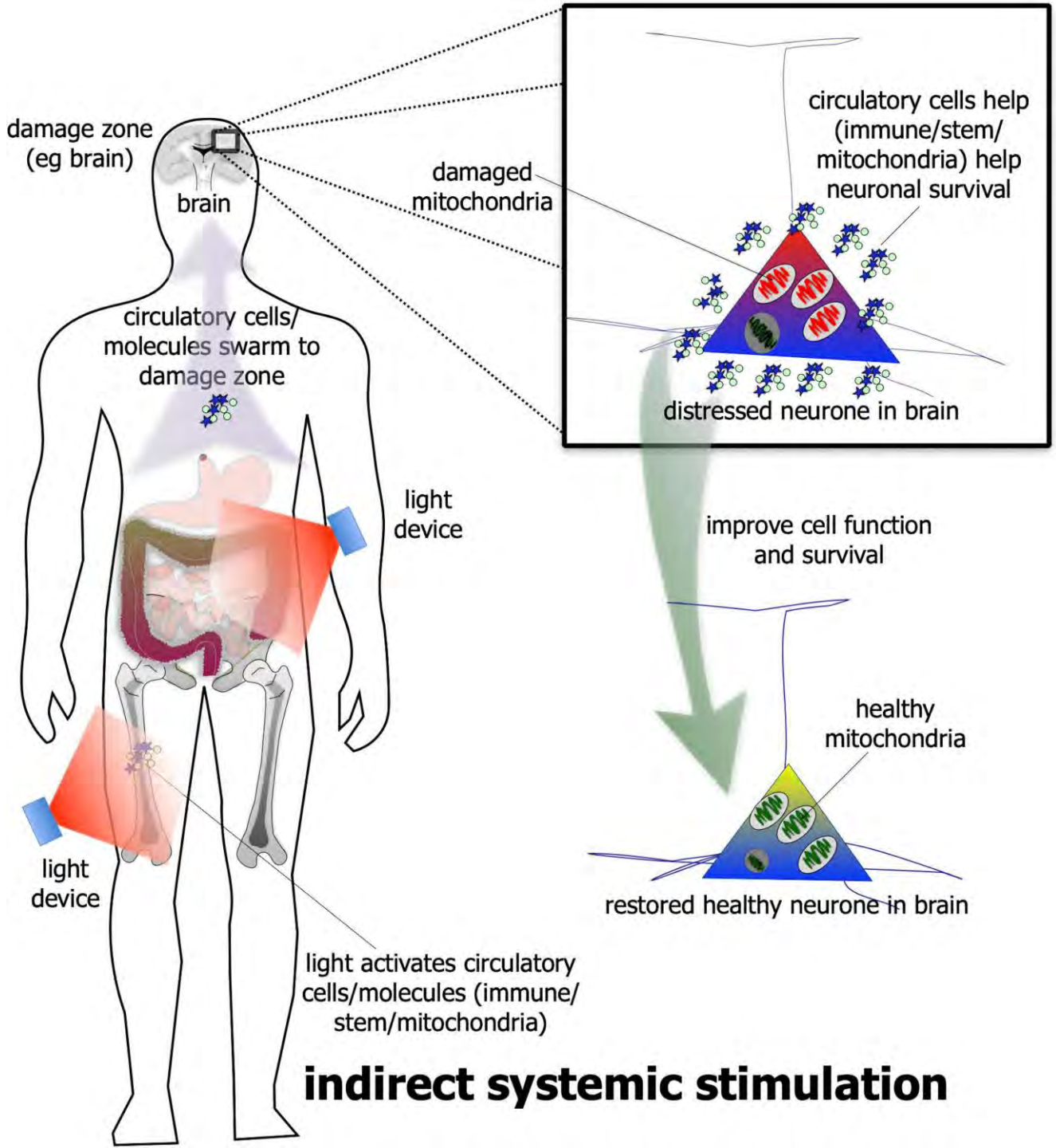
Torre, Jack. (2017). **Treating cognitive impairment with transcranial low-level laser therapy**. *Journal of Photochemistry and Photobiology B: Biology*. 168. 10.1016/j.jphotobiol.2017.02.008.

Schematic diagram showing the direct stimulation mechanism of photobiomodulation



The effect of photobiomodulation on the brain during wakefulness and sleep. *Front Neurosci*, 28 July 2022

Schematic diagram showing the indirect stimulation mechanism of photobiomodulation



The effect of photobiomodulation on the brain during wakefulness and sleep. *Front Neurosci*, 28 July 2022

The effect of photobiostimulation on the brain during wakefulness and sleep

- Wakefulness: studies show PBM improves:
  - Neuronal function
  - Survival in the brain after stimulating mitochondrial activity in neurons
  - Improvement in clinical signs from Alzheimer's to Parkinson's, and depression to TBI
- During sleep: studies show improvements in:
  - Flow of cerebrospinal fluid
  - Clearance of waste from the brain
  - Does so by increasing activity of the glymphatic system
  - Conjecture: increase permeability of aquaporin-4 water channels on the astrocytes

C Moro, A Valverde, M Dole, et al. **The effect of photobiomodulation on the brain during wakefulness and sleep**. *Front Neurosci*, 28 July 2022

Lights at night: PBM to improve sleep

Conclusion:

- Poor quality sleep can severely affect normal day-to-day functioning
- Long-term sleep issues can lead to CV or neurodegenerative disease
- LLLT improves brain function during sleep - stimulates removal of toxic waste products in venous system
- Nocturnal LLLT – by stimulating function of glymphatic system of the brain at night, will form effective non-pharmacological treatment that helps improve overall quality of sleep, hence well-being and long-term health of many individuals
- 635 wavelength shown to promote release of melatonin

A Valverde, C Hamilton, C Moro, et al. **Lights at night: does photobiomodulation improve sleep?**. *Neural Regeneration Research,* March 2023;18(3):474-77

Violet light/ circadian rhythm

- Violet light **strongest** synchronizing agent for circadian rhythm
- Violet light suppresses melatonin during the day

Wahl S, Engelhardt M, Schaupp P, et al. **The inner clock-Blue light sets the human rhythm.** *J Biophotonics*. 2019 Dec;12(12):e201900102

Red light/brain

- Improve clearance of fluid and toxic substances from both periphery and brain
- Periphery – relaxes lymphatic vessels, together with increase in permeability of lymphatic endothelium
- In brain:
  - Shown to reduce beta-amyloid during sleep
  - Also reduces β-amyloid deposition in interstitial space

A Valverde, C Hamilton, C Moro, et al. **Lights at night: does photobiomodulation improve sleep?**. *Neural Regeneration Research,* March 2023;18(3):474-77

Microglia

- **M1** – microglia release inflammatory mediators and induce inflammation and neurotoxicity

- **M2** – microglia release anti-inflammatory mediators and induce anti-inflammatory and neuroplasticity

Microglia Polarization From M1 to M2 in Neurodegenerative Diseases. *Front. Aging Neurosci.*, 16 February 2022;14. *Sec. Neuroinflammation and Neuropathy*

Macrophages

- PBM modulates ratio of M1 & M2 macrophage phenotypes, reducing proinflammatory cytokines and chemokines, increasing anti-inflammatory cytokines, thus balance inflammation process

A Liebert, B Bicknell, W Markman, H Kiat. **A Potential Role for Photobiomodulation Therapy in Disease Treatment and Prevention in the Era of COVID-19**. *Aging and disease*, 2020/12/01;1352(11)

Remote photobiomodulation treatment for the clinical signs of Parkinson's Disease (PD)

Results:

Clinical signs of PD shown to be improved by remote PBM treatment includes:

- Mobility
- Cognition
- Dynamic balance
- Spiral test
- Sense of smell

Liebert A, Bicknell B, Laakso EL, et al. **Remote Photobiomodulation Treatment for the Clinical Signs of Parkinson's Disease: A Case Series Conducted During COVID-19**. *Photobiomodul Photomed Laser Surg*. 2022 Feb;40(2):112-122

LLLT/Parkinson's disease

Results:

- Statistical significant reduction in VAS for gait and cognitive function were observed
- Gait – 30% improvement
- Cognitive – 38% improvement
- Difficulty with speech lowered by study end

Conclusion:

Data suggest laser therapy may serve as non-invasive instrument for symptom reduction of PD

Erchonia Medical. **The Application of Low-Level Laser Therapy for the Symptomatic Care of Late Stage Parkinson's Disease: A Non-Controlled, Non-Randomized Study**. *Lasers Surg Med* , 22 Sept 2022;185

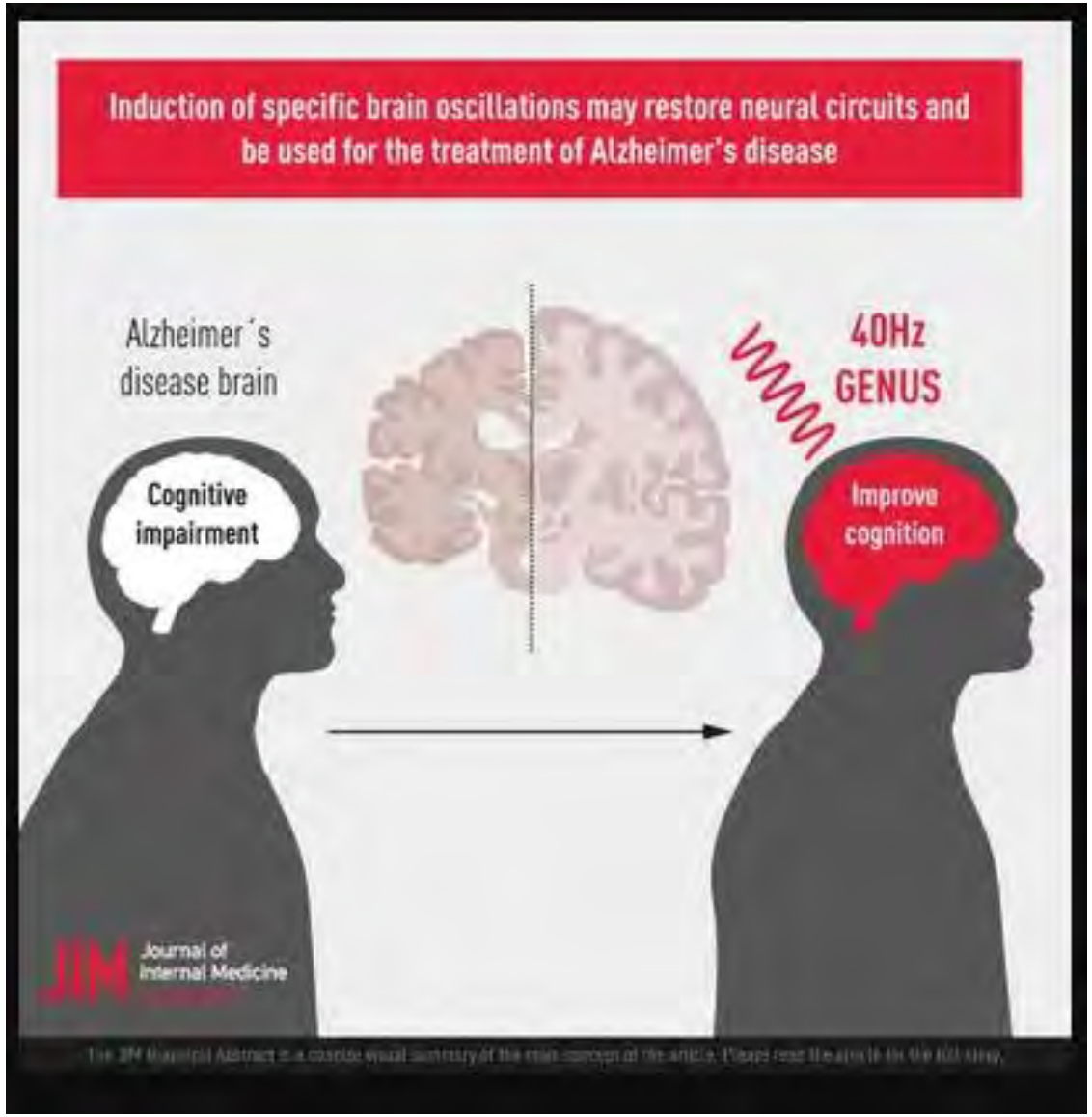
Alzheimer’s disease

- LLLT can directly disassemble amyloid-beta in vitro and in vivo
- Activates mitochondrial cytochrome-c to produce ATP in the neurons
- A promising strategy combined LLLT with nanopacked Q10 proposed to apply for treating AD

Tian Z, Wang P, Huang K, et al. **Photobiomodulation for Alzheimer's disease: photoelectric coupling effect on attenuating Aβ neurotoxicity.** *Lasers Med Sci*. 2023 Jan 12;38(1):39

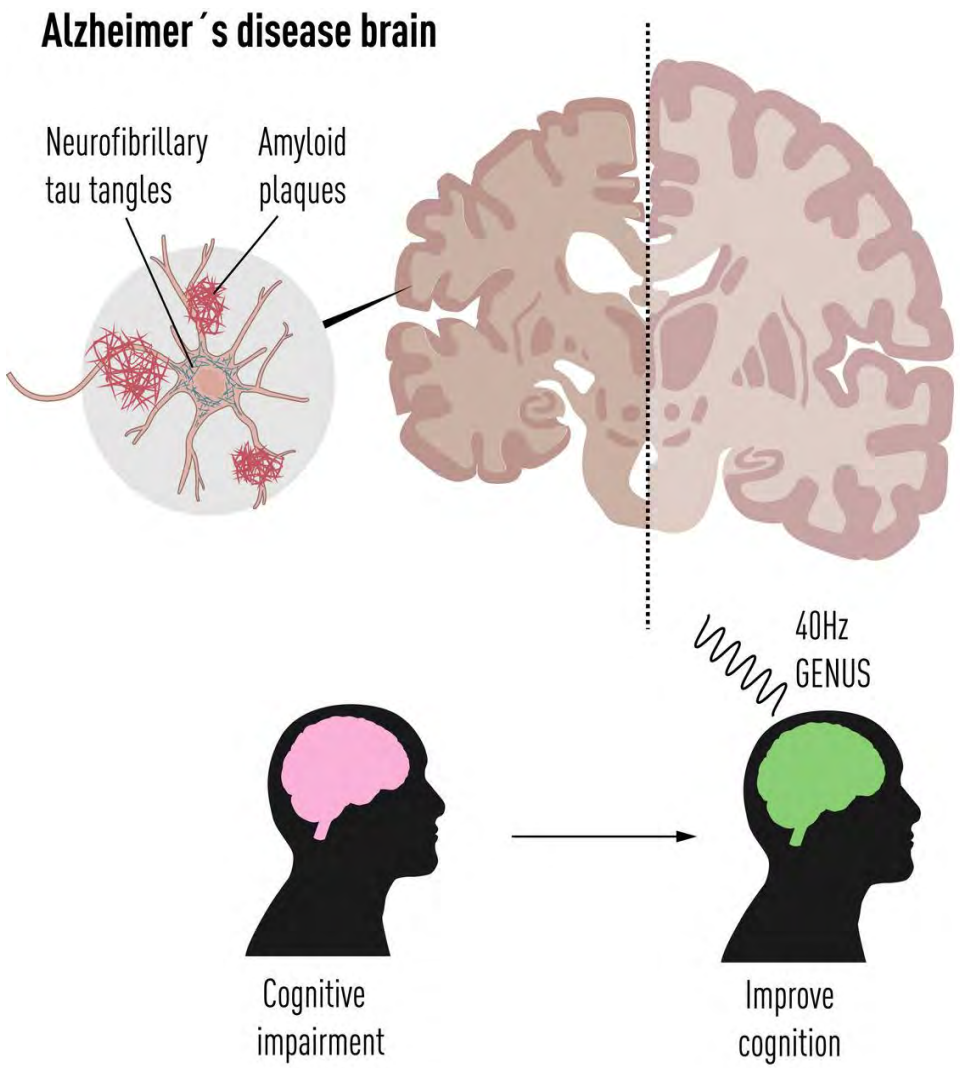
40 Hz transcranial alternating current stimulation

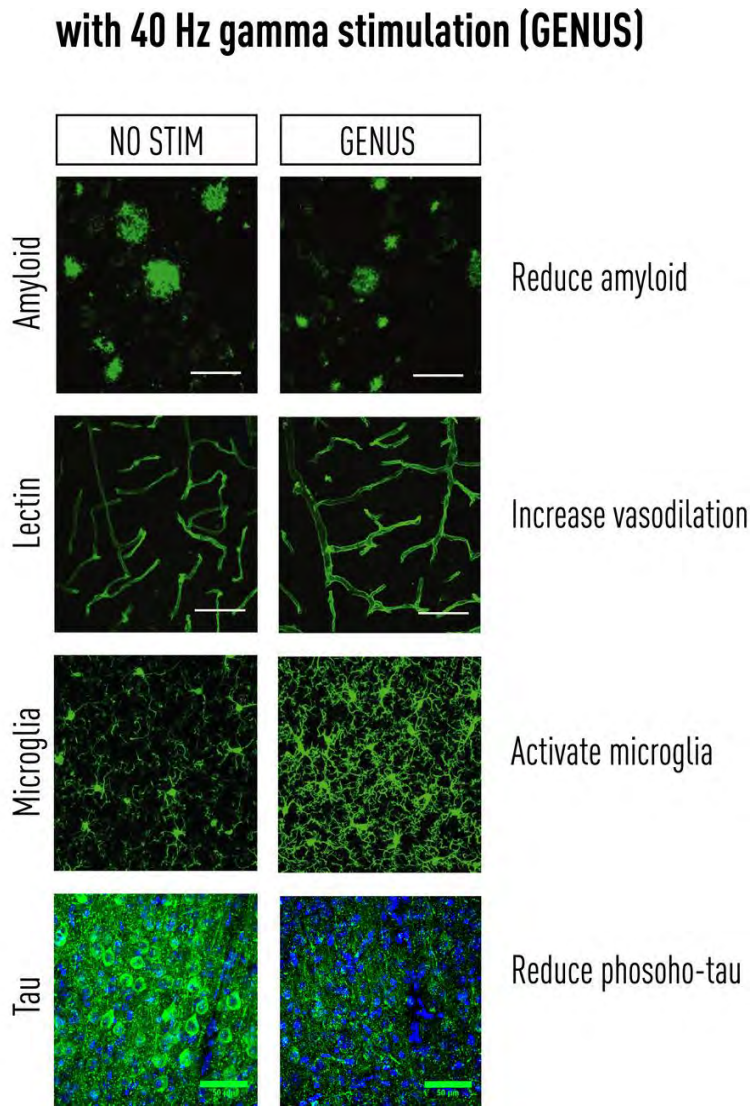
- Exogenously-induced 40 Hz gamma oscillations – reduced amyloid-β and p-TAU deposition presumably via microglia activation



Dhaynaut M, Sprugnoli G, Cappon D, et al. **Impact of 40 Hz Transcranial Alternating Current Stimulation on Cerebral Tau Burden in Patients with Alzheimer's Disease: A Case Series**. *J Alzheimers Dis*. 2022;85(4):1667-1676

Effects of 40-Hz light and sound GENUS on the AD brain





Induction of specific brain oscillations may restore neural circuits and be used for the treatment of Alzheimer's disease. J of Int Med, 22 June 2021;290(5):993-1009

Photobiomodulation (PBM) in Alzheimer's disease

Results:

Studies showed PBM able to reduce inflammatory response, oxidative stress and apoptotic effects generated by amyloid beta and restore mitochondrial function/cognitive behavior

Conclusion:

Results indicate PBM maybe be useful tool for treating AD

Cardoso FDS, Lopes Martins RÁB, Gomes da Silva S. **Therapeutic Potential of Photobiomodulation In Alzheimer's Disease: A Systematic Review**. *J Lasers Med Sci*. 2020 Fall;11(Suppl 1):S16-S22

PBM/exercise/Alzheimer's disease

Findings:

The experimental group which received active LLLT in addition to moderate-intensity aerobic exercise showed more significant results compared to control group which received placebo LLLT and moderate-intensity aerobic exercise

Nagy EN, Ali AY, Behiry ME, et al. **Impact of Combined Photo-Biomodulation and Aerobic Exercise on Cognitive Function and Quality-of-Life in Elderly Alzheimer Patients with Anemia: A Randomized Clinical Trial**. *Int J Gen Med*. 2021;14:141-152

LLLT ameliorates disease progression in a mouse model of Alzheimer's disease (AD)

Conclusion: Results suggest use of LLLT as a therapeutic application in progressive stages of AD

J Mol Neurosci 2015 Feb; 55(2):430-6

LLLT for beta amyloid toxicity

Conclusion:

By alleviating a broad spectrum of AB-induced pathology that includes mitochondrial dysfunction, oxidative stress neuroinflammation, neuronal apoptosis, and tau pathology, LLLT represents a new promising therapeutic strategy for AD

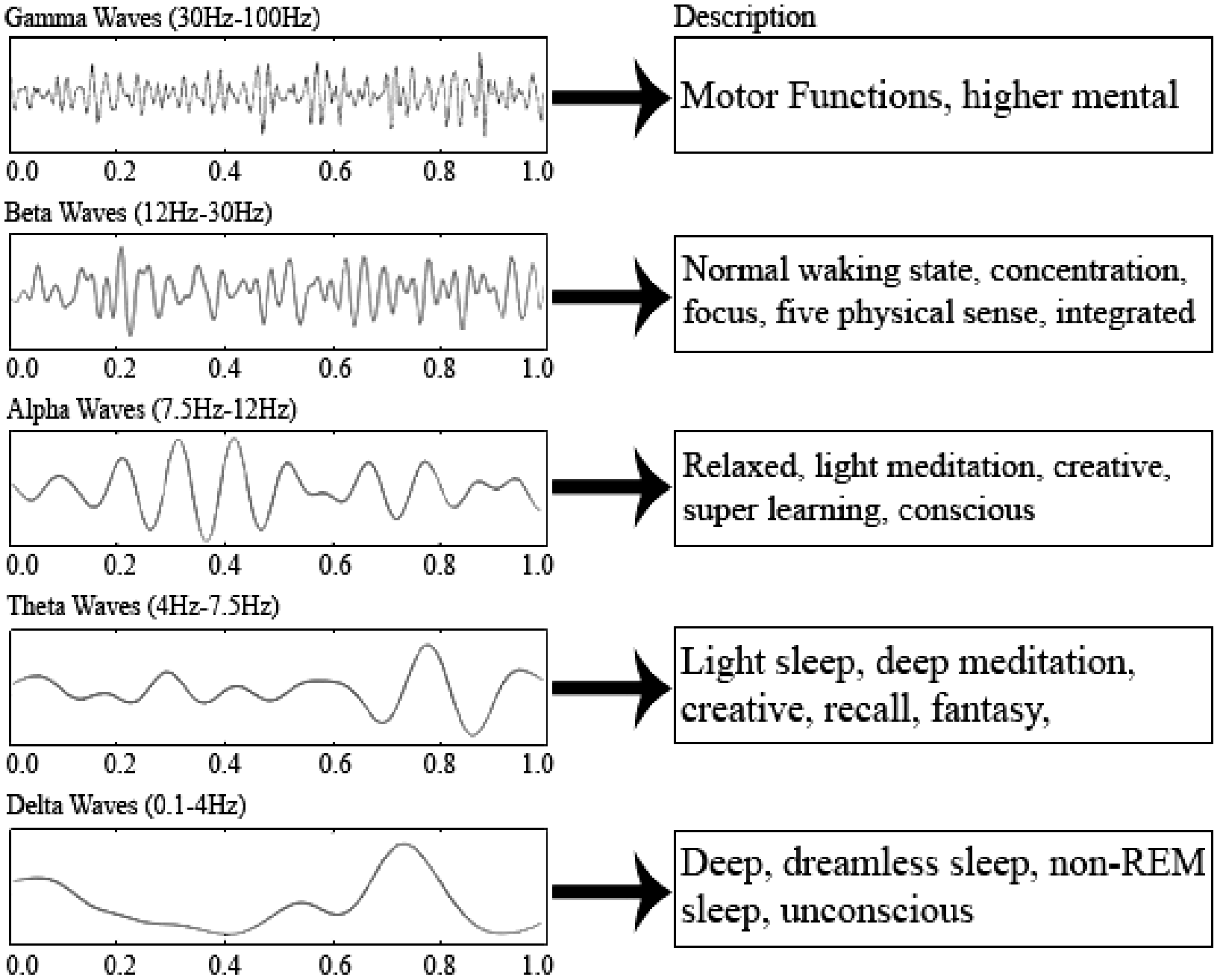
Photobiomodulation therapy and Alzheimer's

Findings:

Gf (gut flora)-targeted PBM regulates diversity of intestinal flora, which may improve damage caused by AD. Gf-targeted PBM has potential to be noninvasive microflora regulation method for AD patients

Qianqian C, Jinpeng W, Xiaoxi D, et al. **Gut flora-targeted photobiomodulation therapy improves senile dementia in an Aß-induced Alzheimer's disease animal model**, *J of Photochemistry and Photobiology B: Biology*, 2021;216:112152

Brain waves charts description



W Srimaharaj, R Chaisricharoen, S Chaising, P Sittiprapaporn (2018). **Classification of human brain attention focused on meditation, effected by L-theanine acid in Oolong tea**. *Conference: 2018 International Conference on Digital Arts, Media and Technology (ICDAMT)*

Alzheimer’s disease

- Genetics – ApoE4
- Hormonal status
- Gut status
- Inflammatory status
- Oral microbiome
- Sinusitis
- Molds air pollution

Mark Hyman podcast. 15 Dec 2021, Ep. 401

4 stages of Alzheimer's disease

Stages to treat for best outcomes:

- Asymptomatic – abnormal spinal fluid, abnormal pet scan
- Subjective cognitive impairment – lasts for 10 years

- Mild cognitive impairment – should be called relatively advanced Alzheimer's disease (late-stage of the problem)
- Alzheimer's disease

Mark Hyman podcast. 15 Dec 2021, Ep. 401

Alzheimer’s disease factoids

- Amyloid – anti-microbial agent made in response to inflammation
- Cognitive decline with Alzheimer’s causes grey matter volume to shrink 4.5 x annually
- Normal aging people lose 1.7 x grey matter volume
- You need to be keto-adapted and metabolically flexible

Mark Hyman podcast. 15 Dec 2021, Ep. 401

ApoE

- ApoE gene on chromosome 19
- Encodes instructions for making protein that helps transport cholesterol and other types of fat in bloodstream
- 3 main focus:
  - ApoE2 – relatively rare. If inherited – protective of developing Alzheimer's
  - ApoE3 – most common allele, no real effect
  - ApoE4 – increases risk of Alzheimer's

Erin B. **Study reveals how APOE4 gene may increase risk for dementia**. *NIH Research Matters*, 16 March 2021

APOe is associated with disease progression in various conditions

Role of apolipoprotein E in neurodegenerative diseases. *Neuropsychiatric disease and treatment,* July 2015;11:1723-37

ApoE4/BBB/TBI

Findings:

- ApoE3, ApoE4 have similar acute BBB responses to TBI
- ApoE3 displays faster spontaneous BBB repair than ApoE4
- Highlights ApoE4 allele as risk factor for poor outcomes after TBI

BS Main, S Villapol, SS Sloley, *et al.* **Apolipoprotein E4 impairs spontaneous blood brain barrier repair following traumatic brain injury**. *Mol Neurodegeneration,* 4 Apr 2018;13:17

What's the problem? Alzheimer's disease

- 45 million Americans will die from Alzheimer's disease without protection
- Greatest "failure" of biomedical research
- Core of Alzheimer's – fundamentally a "network insufficiency"
- Perfect disease for functional medicine practitioner
- Systems biology disorder
- Prevent and reverse decline
- Whole body problem

Mark Hyman podcast. 15 Dec 2021, Ep. 401

What's the problem? Alzheimer's disease (cont'd)

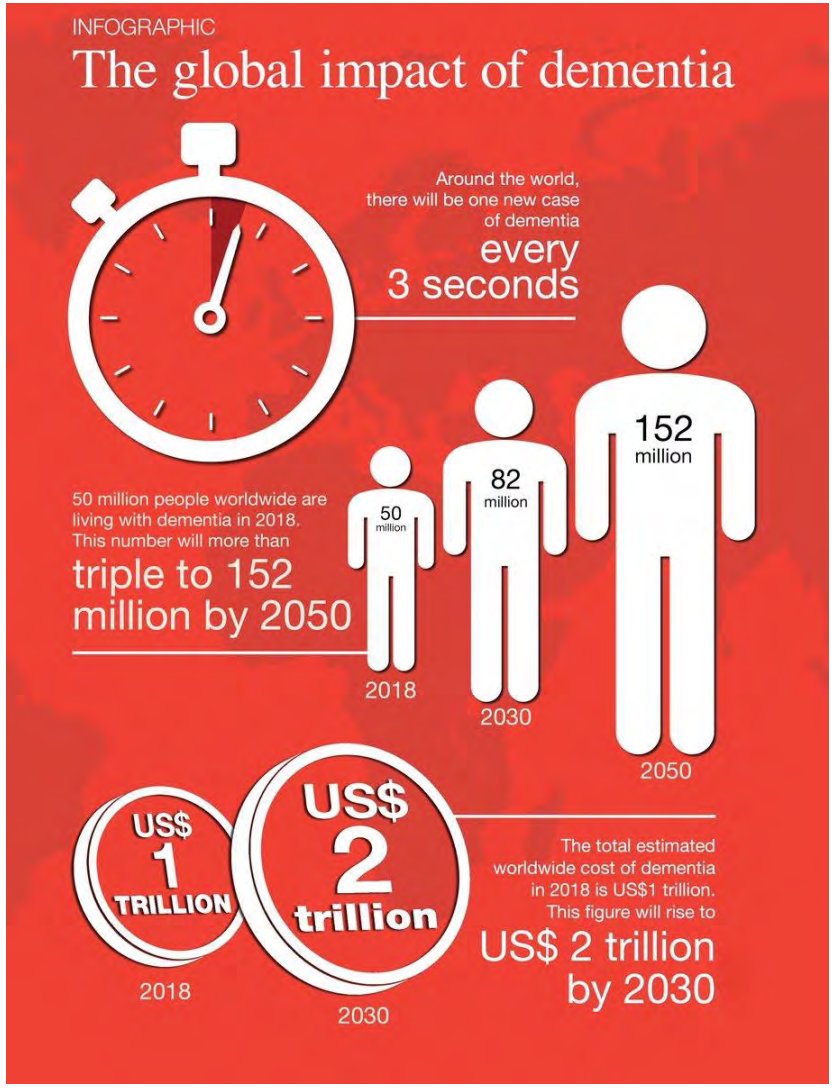
Causes:

- Insulin resistance
- Inflammation
- Lack of trophic factors – things that help the brain work
- Toxins
- Energetics

Mark Hyman podcast. 15 Dec 2021, Ep. 401

ALZHEIMER'S DISEASE

- **6th leading cause of death** in the US; **7th** in the world
- 2000-2015: heart attack deaths decreased 11%; Alzheimer's deaths **increased** 123%
- 1 in 3 seniors die from Alzheimer's/dementia – kills more than breast and prostate cancer combined
- 2018 – Alzheimer's/dementia (US) cost $277 billion
- By 2050 – Alzheimer's/dementia (US) could cost **> $1.1 trillion**
- Someone in the US develops the disease every **65 seconds**



Credit: *World Alzheimer's Report 2018*

Alzheimer's Association 2018 Alzheimer's Disease Facts and Figures
World Alzheimer's Report 2018

Women's brains in Alzheimer's Disease

- 60-70% of Alzheimer's sufferers are women
- Women with MCI found to decline faster than men with similar diagnosis
- Similar levels of biomarkers might have different prognostic values for men and women
- Potential female risk factors:
  - Ovariectomy
  - Hypertensive complications during pregnancy
  - Number of pregnancies
- Mounting evidence indicating microglial cells are different in women than men

Nature Reviews Neurology, 9 July 2018:457-69

P. gingivalis in Alzheimer's disease

- *P. gingivalis* found in brains of Alzheimer's patients
- Bacteria creates destructive enzymes – gingipains
- Infiltrates brain and causes inflamed damage
- Over 90% of Alzheimer's disease samples had gingipains
- Also identified in CSF

Science Advances. 23 Jan 2019;5(1)

Lab tests for brain health

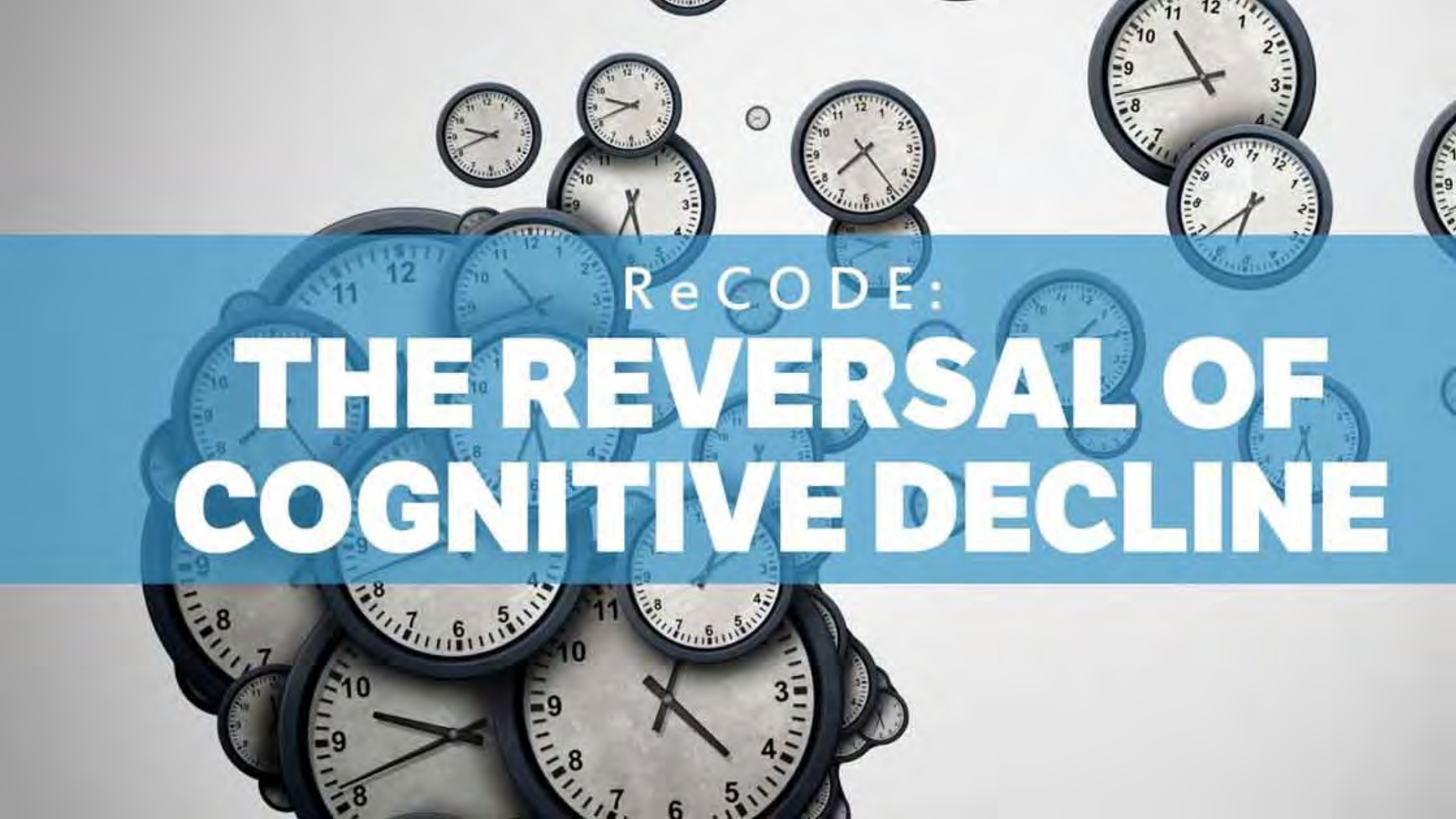
- Fasting blood glucose
- Hemoglobin A1c
- Fasting insulin
- Homocysteine
- C-reactive protein
- Vitamin D

Grain Brain, 2018:25-27

ALZHEIMER'S BLOOD TEST

- Blood test for a protein (NFI)
- Neurofilament light chain
- Normally resides inside brain cells
- When damaged, dying cells leak into CSF
- Protein then travels into bloodstream
- NFL levels predict symptoms 16 years ahead

Nature Medicine. Jan 21, 2019;25:277-83

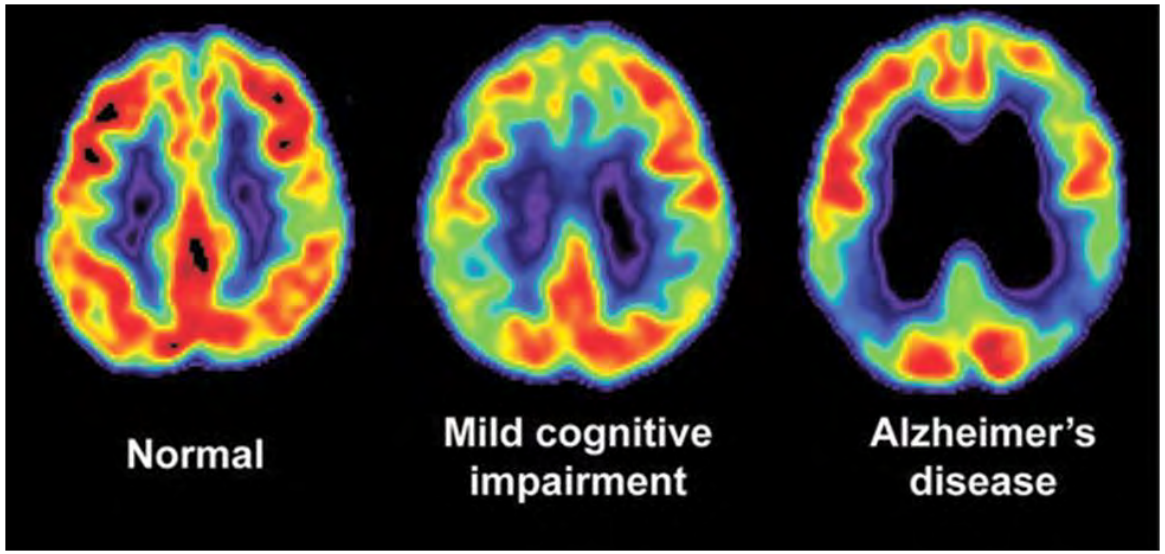


ReCODE:

THE REVERSAL OF COGNITIVE DECLINE

Alzheimer's protocols

- Check for ApoE e4 gene
- Increase BDNF
- Keto or Mediterranean diets
- Plant-based diets
- Intermittent fasting
- Choose foods with GI under 35
- GPS/DNA
- Pre and probiotics/heal the gut
- Cook at proper temperatures and times
- Lifestyle changes



ANTI-ALZHEIMER'S DIET: KETOFLEX 12/3

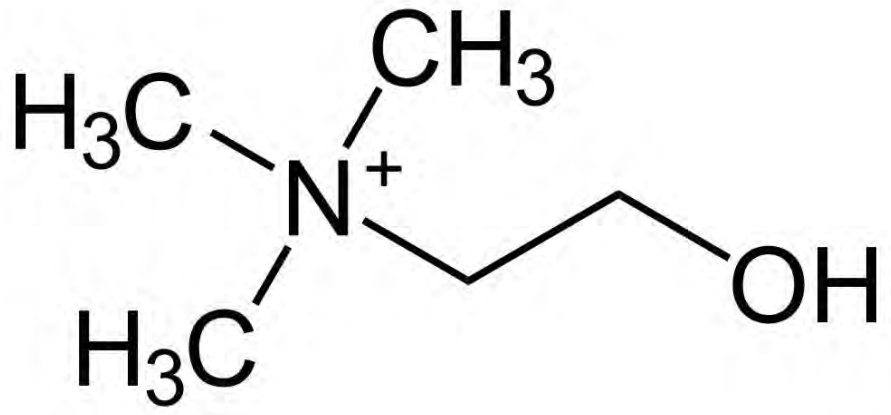
- Include supplements:
  - Methylated B vitamins
  - Vitamin C – 1 g
  - Vitamin D – 5000 IU
  - Vitamin E – mixed tocopherols – 600 IU
  - Vitamin K2 (MK-4 and MK-7) – 100 mcg
  - Resveratrol – 100 mg
  - Acetyl-L-carnitine – 500 mg
  - Co-enzyme Q10 – 200 mg
  - Omega-3 FA – 2-4 g
  - Curcumin – 1 g
  - Pro-resolving Mediators – 1000 mcg
  - Mg – 400-600 mg

Adapted from *The End of Alzheimer's*, Dr. Dale Bredesen

Choline for Alzheimer's Disease

In new study: A life-long dietary regiment of choline holds potential to revert Alzheimer's disease

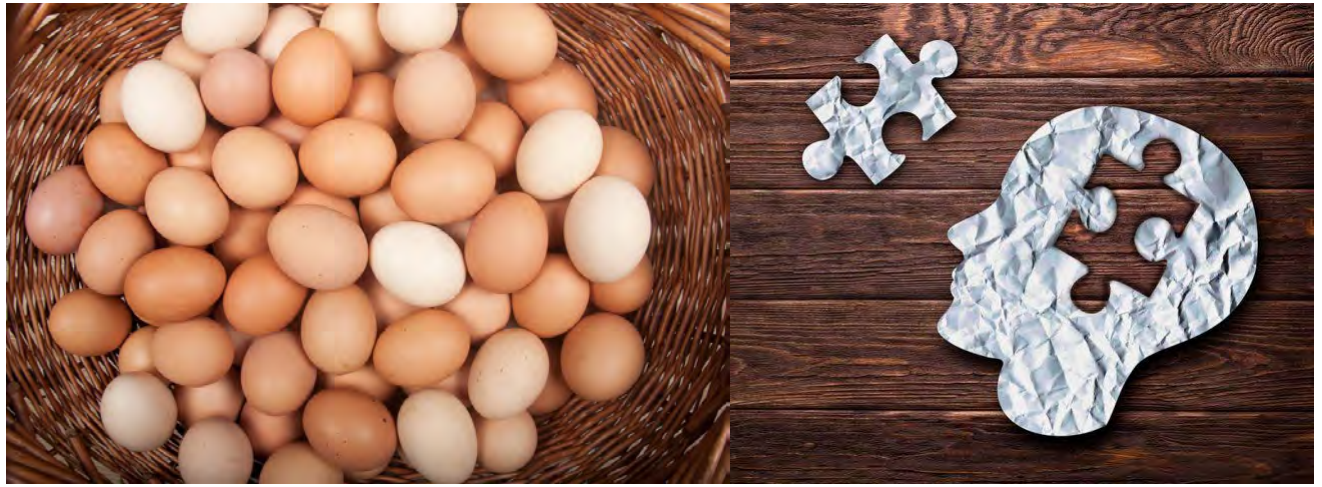
- Benefits of choline supplementation reduce activation of microglial
- Reduction in microglial suggests ways of treating broad range of disorders – TBI, Parkinson's disease, multiple sclerosis



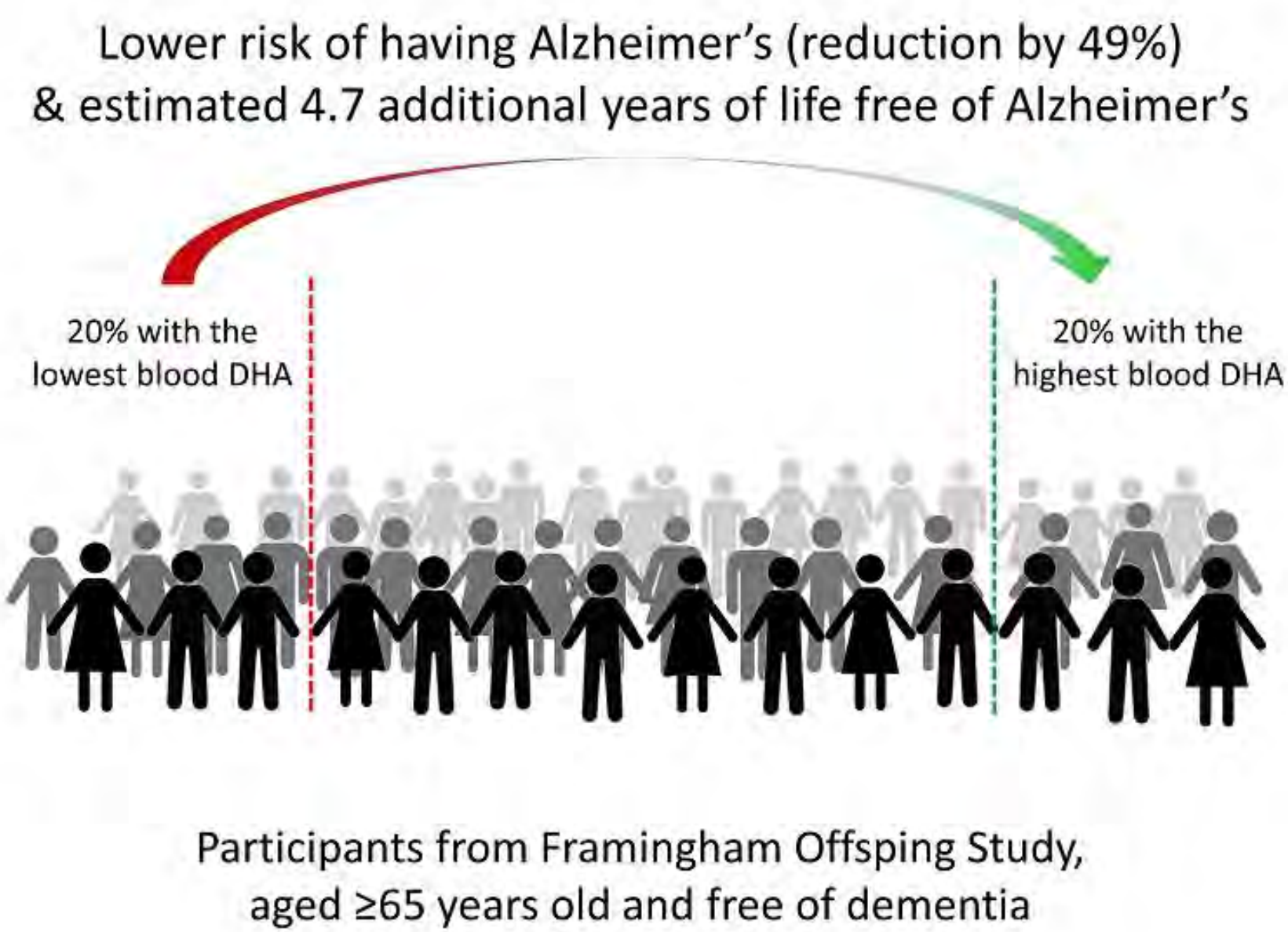
R. Velazquez, E. Ferreira, S. Knowles, et al. *Aging Cell*, 2019

Choline for Alzheimer's Disease

- Choline blocks production of amyloid-beta plaques
- Choline decreases microglial mechanistically – alters:
  - Alpha7 nicotinic acetylcholine
  - Sigma-1 receptors (agonist)



R. Velazquez, E. Ferreira, S. Knowles, et al. *Aging Cell*, 2019



Red Blood Cell DHA Is Inversely Associated with Risk of Incident Alzheimer's Disease and All-Cause Dementia: Framingham Offspring Study. *Nutrients*. 2022; 14(12):2408

Lifestyle changes

- Exercise
- Sleep
- Reduce stress
- Brain training
- Resolve inflammation
- Inhibit new inflammation
- Remove all inflammatory sources
- Heal the gut – 7R Program

Adapted from *The End of Alzheimer's*, Dr. Dale Bredesen

Sleep/Alzheimer's disease

When young healthy men were deprived of 1 night of sleep – had higher levels of tau (biomarker for Alzheimer's disease) in their blood than when they had full night of rest

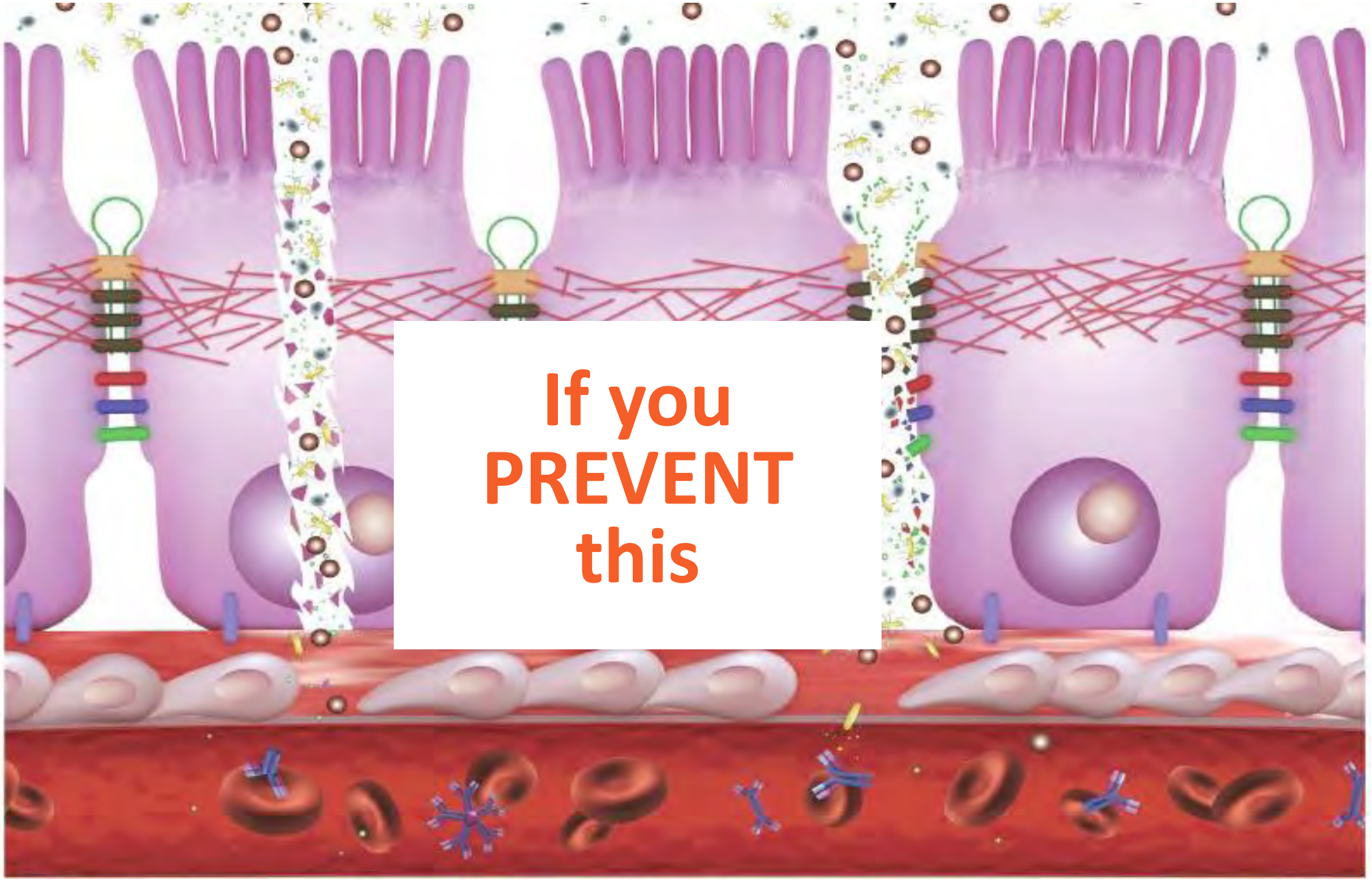
Christian B, Kaj B, Henrik Z, et al. **Effects of acute sleep loss on diurnal plasma dynamics of CNS health biomarkers in young men,** *Neurology* Jan 2020. Online

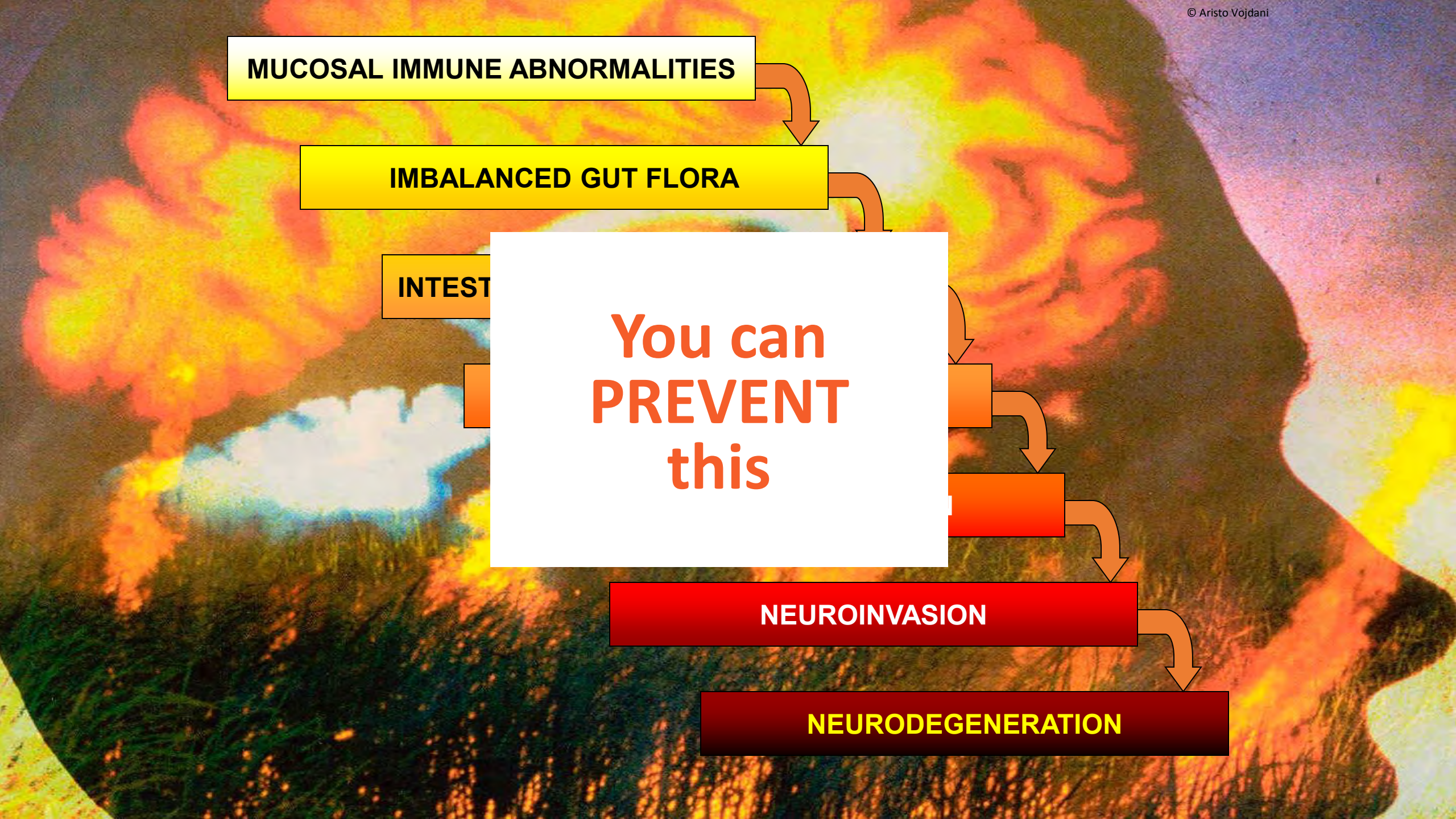
Alzheimer’s protocol

- Dynamic Multi Powder: 1 scp daily
- Dynamic Brain Restore: 1 scp daily
- D3 10,000 with K2: 1 sg daily
- PRM Resolve: 2 sg BID
- Curcumin 400X: 2 sg daily

Alzheimer’s protocol (cont’d)

- Magtein: 3 caps daily
- Omega Pure EPA-DHA 1000: 2 sg BID
- CoQ10 Ubiquinol: 3 sg daily
- Resveratrol Plus: 2 sg daily
- MCT Oil: 2 tsp daily

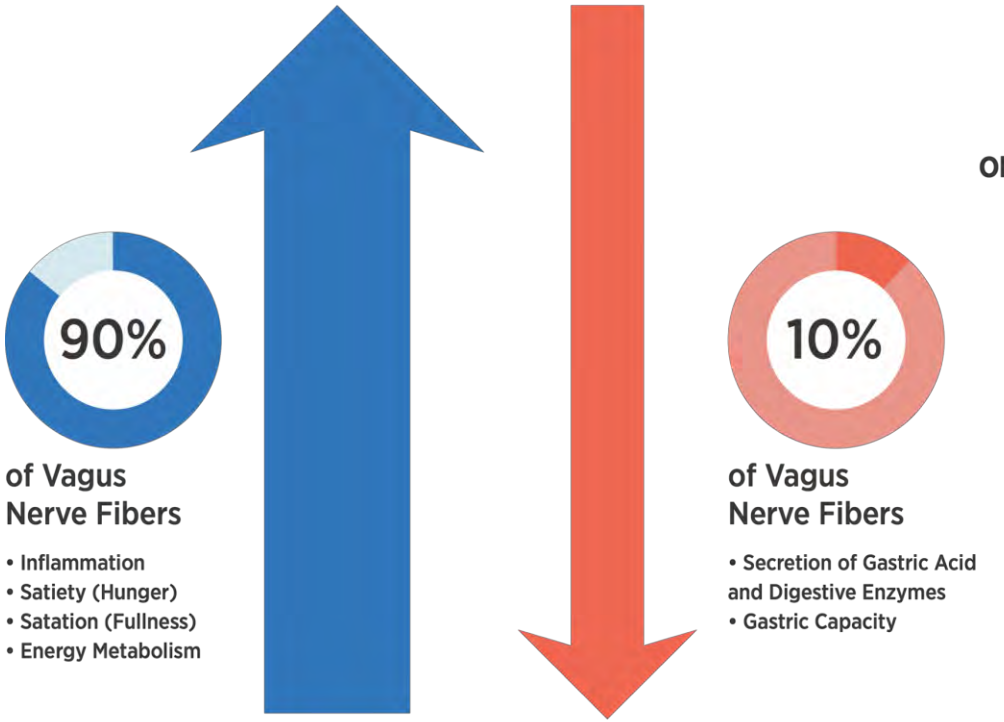

If you
PREVENT
this


© Aristo Vojdani
MUCOSAL IMMUNE ABNORMALITIES
IMBALANCED GUT FLORA
INTEST
You can PREVENT this
NEUROINVASION
NEURODEGENERATION

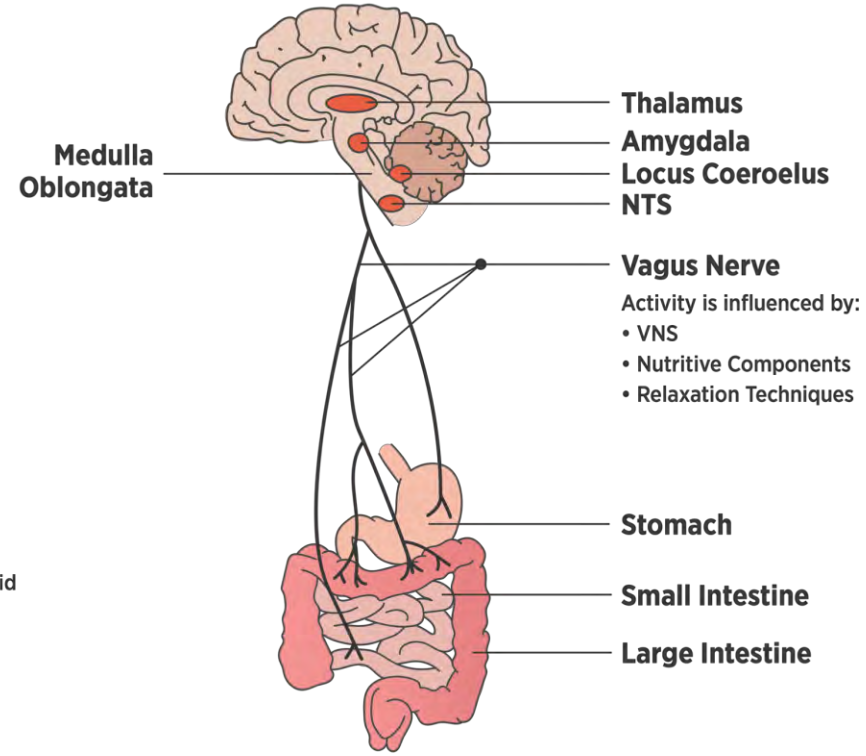

NEXT
BIG THING
AHEAD

BASIC ANATOMY AND FUNCTIONS OF THE VAGUS NERVE

AFFERENT & EFFERENT CONNECTIONS



ANATOMY



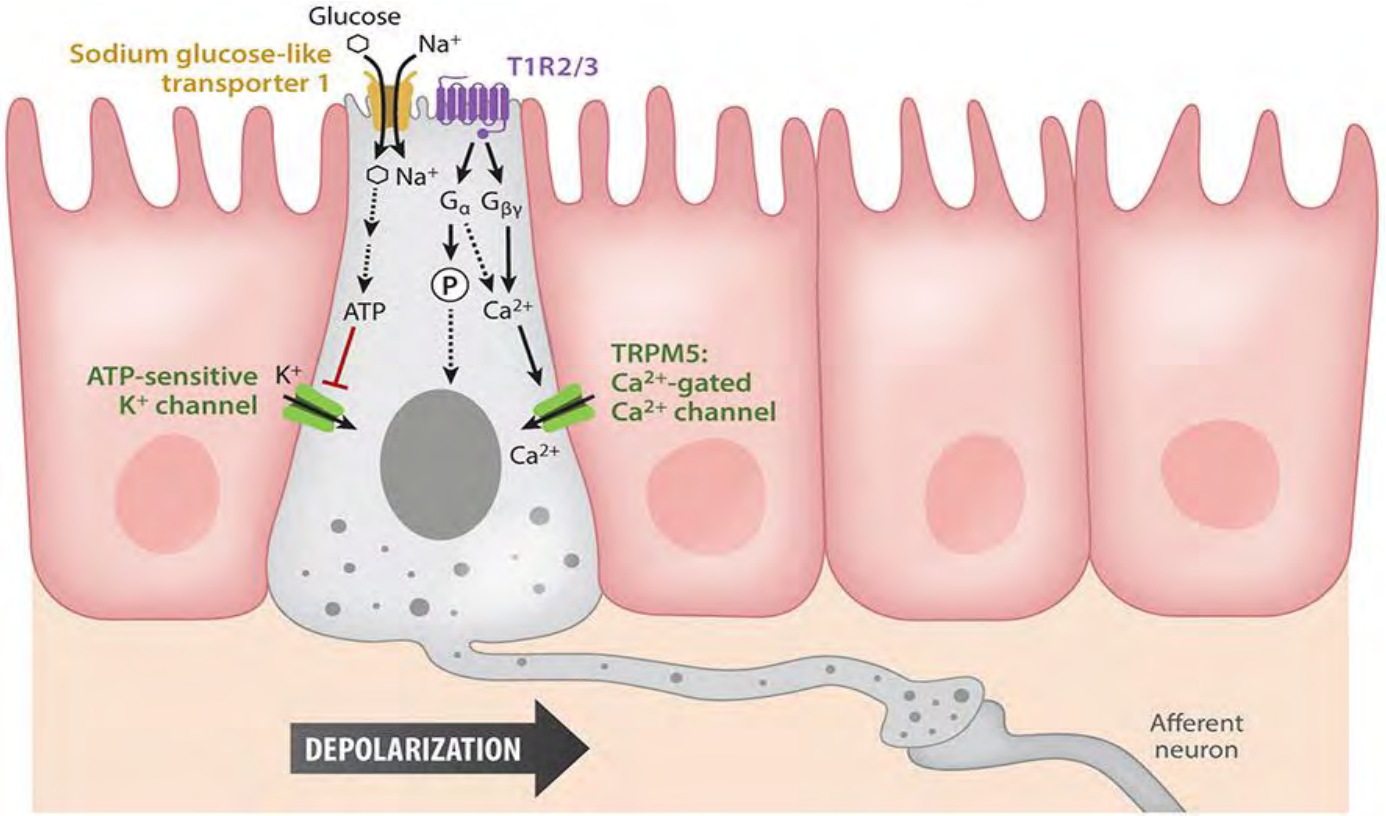
DISORDERS

Psychiatric Disorders
- Major Depression
- PTSD

Inflammatory GI Disorders
- Ulcerative Colitis
- Crohn's Disease

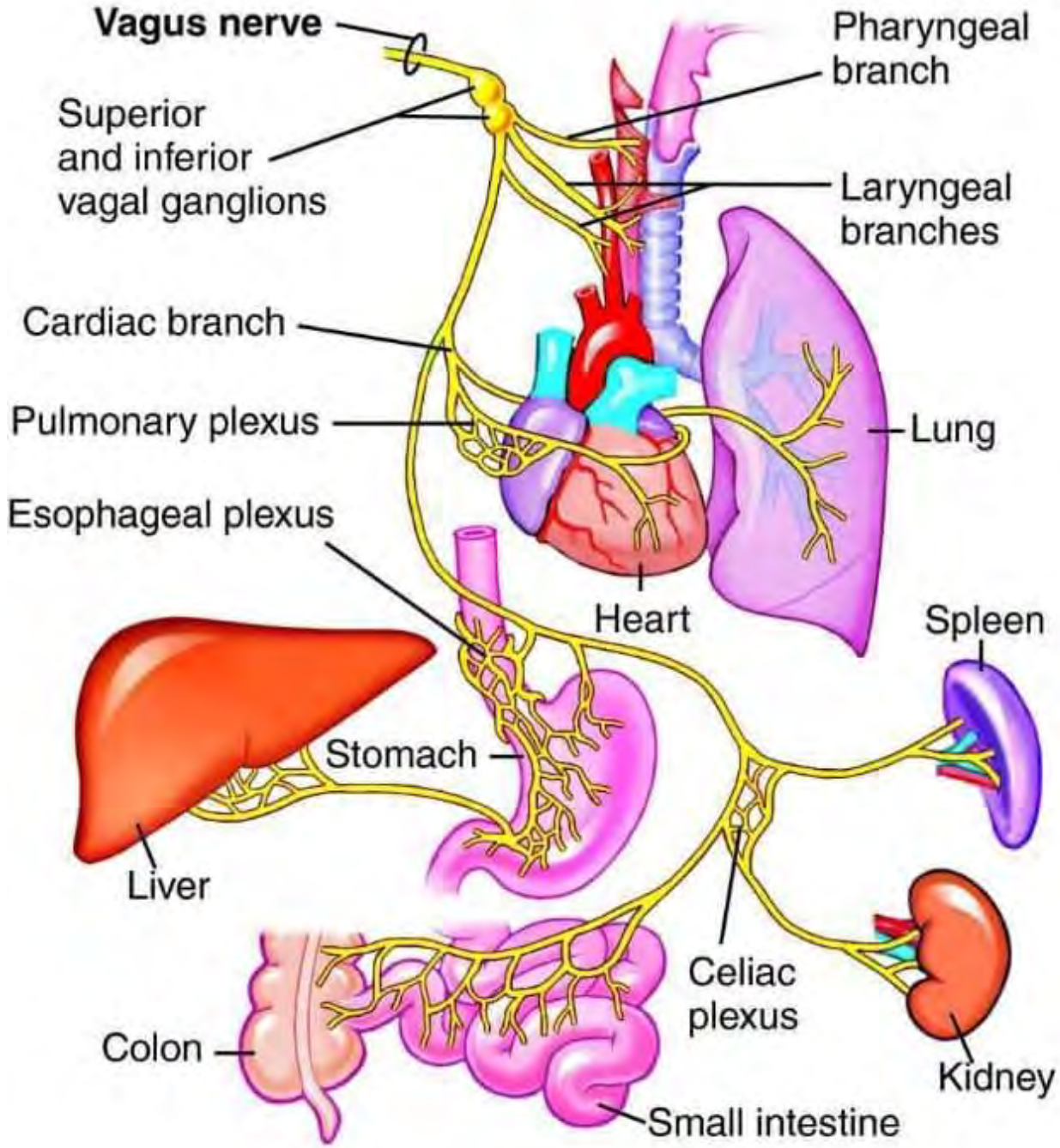
Frontiers in NeuroScience. Feb 7, 2018;12:1-8

Vagus nerve/neuropod cell



Neuropod cells provide foundation for gut to transduce sensory signals from the intestinal milieu to the brain through fast neurotransmission onto neurons including those of the vagus nerve

Kaelberer MM, Rupprecht LE, Liu WW, et al. **Neuropod Cells: The Emerging Biology of Gut-Brain Sensory Transduction**. *Annu Rev Neurosci*. 2020 Jul 8;43:337-353

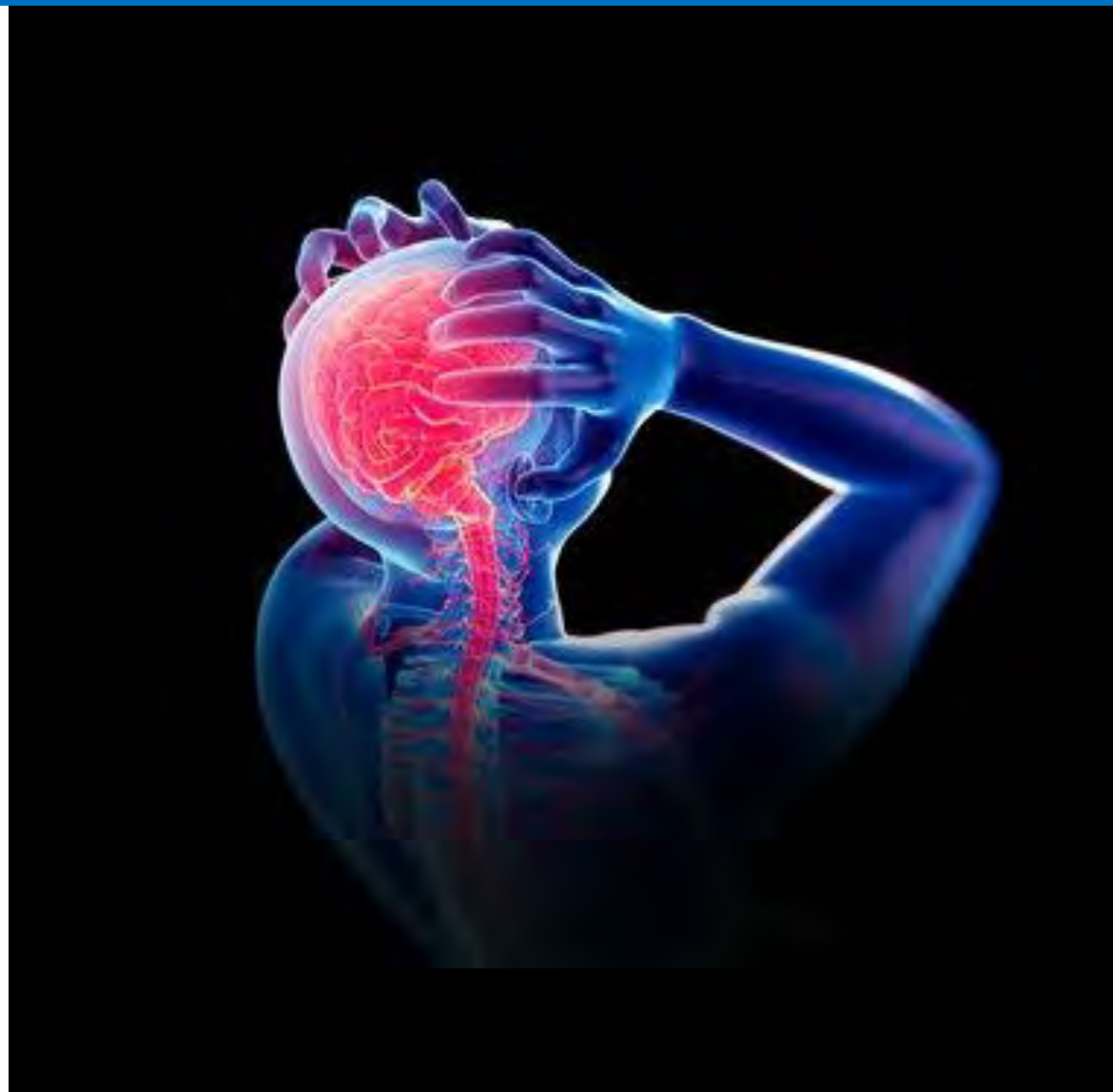

Vagus nerve
Superior and inferior vagal ganglions
Pharyngeal branch
Laryngeal branches
Cardiac branch
Pulmonary plexus
Lung
Esophageal plexus
Heart
Spleen
Stomach
Liver
Celiac plexus
Kidney
Colon
Small intestine

Vagus nerve
Primary functions

- Parasympathetic: responsible for operation of the digestive tract, heart rate, and respiration
- Special sensory: provides sensations of taste from behind the back of tongue
- Sensory: operating specific mechanisms of the abdomen, heart, lung, throat
- Motor: enabling the movement of neck muscles, enabling speech and swallowing

TBI - VAGUS

- Vagus nerve “rest and digest”
- PNS and SNS cannot both be dominant at the same time
- Following TBI patients find themselves in sympathetic state dominance:
  - Shutting down PNS
  - Affecting normal functionality of vagus nerve
  - Slowing digestion dramatically through mechanism of **MMC**



Frontiers in NeuroScience. Feb 7, 2018;12:1-8

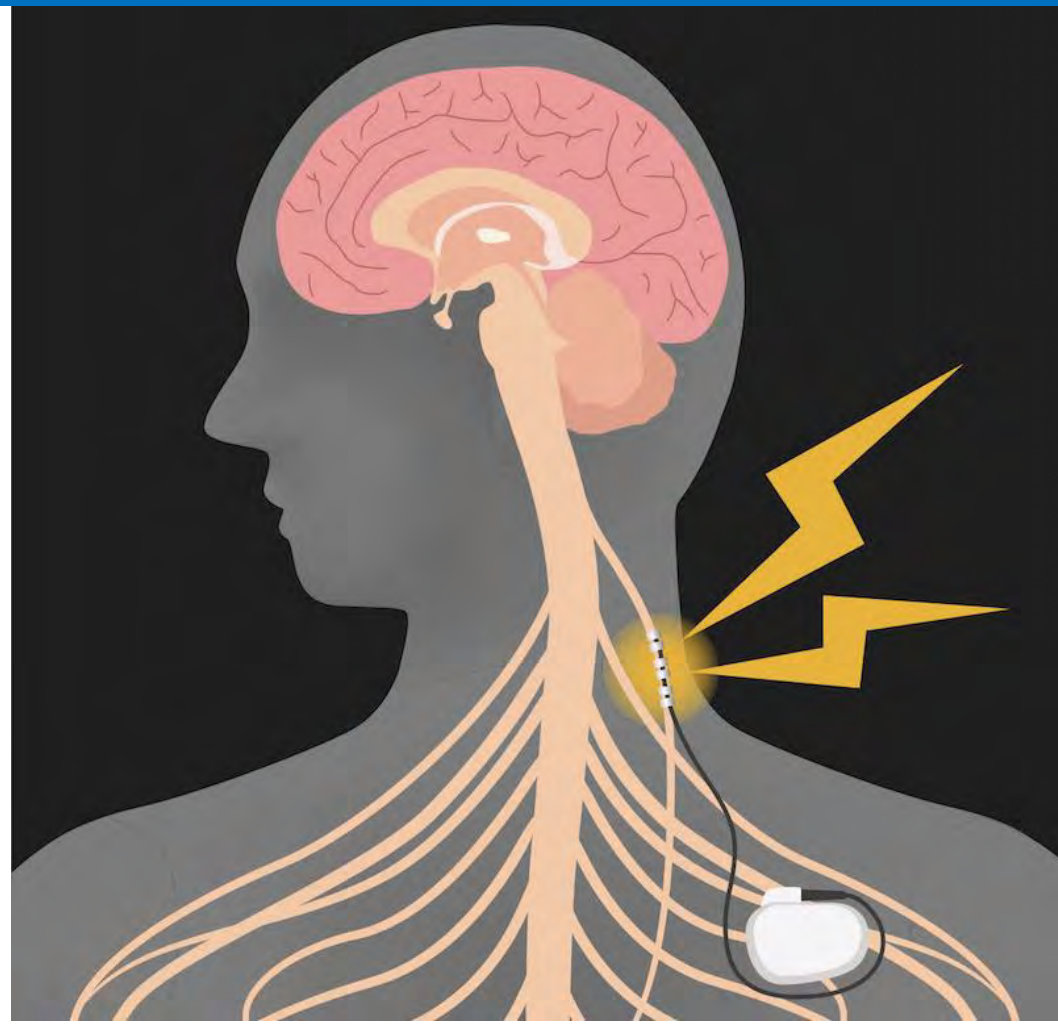
VAGUS NERVE AT THE INTERFACE OF MICROBIOTA-GUT-BRAIN AXIS

- A low vagal tone:
  - Seen in IBS and IBD thus favoring peripheral inflammation
  - Decreased secretion of hydrochloric acid
  - Decreased secretion of pancreatic enzymes
  - Reduced activity of parietal cells
  - Reduced bile secretion
- Targeting the VN through stimulation would be of interest to restore homeostasis in the microbiota-gut-brain axis

Frontiers in NeuroScience. Feb 7, 2018;12:1-8

VAGUS NERVE STIMULATION DRAMATICALLY REDUCES INFLAMMATION

- Stimulating vagus nerve:
  - Acetylcholine
  - Reduces inflammation
  - Improves outcomes in RA
  - Inhibits cytokine production



Psychology Today. July 6, 2016

A neural circuit for gut-induced reward

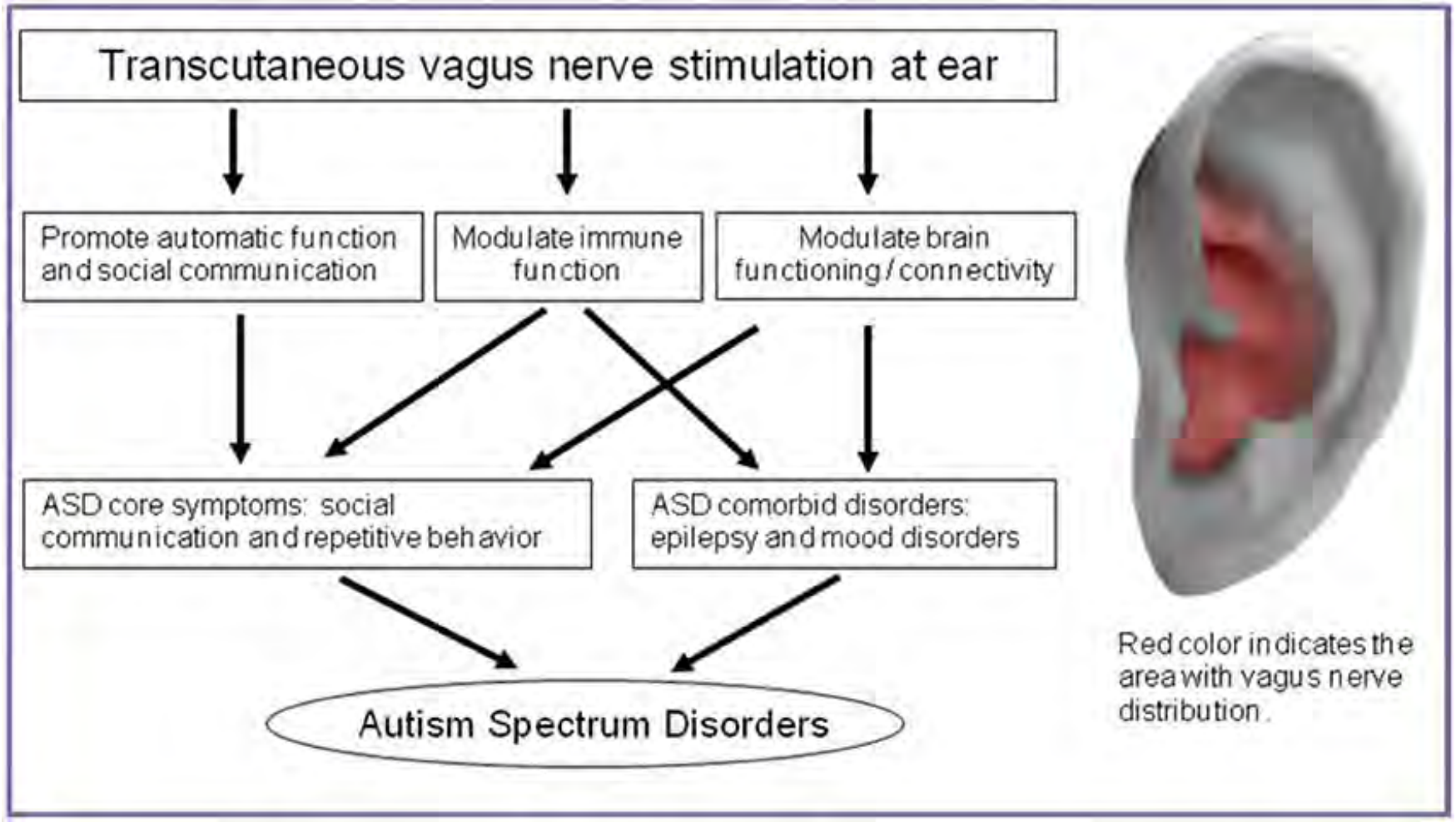
- Critical role for the vagal gut-brain axis in motivation and reward
- Optogenetic stimulation of the vagal gut-brain axis produces reward behaviors (right vagus only)
- Asymmetric brain pathways of vagal origin mediate motivation and dopamine activity (right vagus only)
- Gut-innervating vagal sensory neurons are major components of the reward circuitry (right vagus)
- Left vagus – satiety, not reward

Wenfei H, Luis AT, Matthew HP, et al. *Cell.* Sept. 20, 2018

L. reuteri and Autism

- *L. reuteri* has successfully reversed deficits in social behavior associated with autism
- Works via a mechanism that involves the vagus nerve and oxytocin-dopamine reward system
- When vagus nerve was severed, *L. reuteri* effect was rendered ineffective

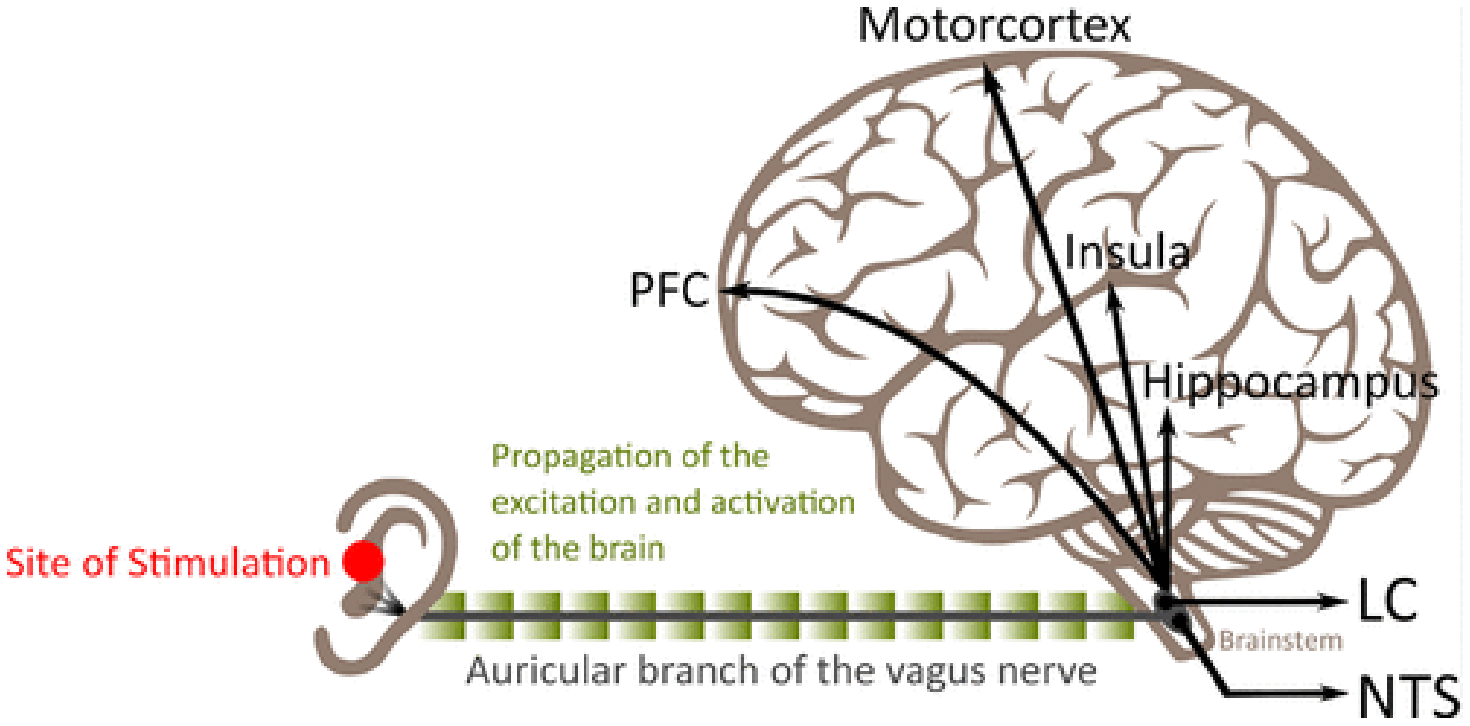
Martina Sgritta, et al. *Mechanisms Underlying Microbial-Mediated Changes in Social Behavior in Mouse Models of Autism Spectrum Disorder, Neuron* 2018



Action mechanism of tVNS on treatment for ASD and the location of vagus nerve distribution in ear

Frontiers in Neuroscience, 20 January 2017

Schematic illustration of the main brain areas involved in the afferent stimulation of the auricular branch of the vagus nerve



https://journals.physiology.org/doi/full/10.1152/jn.00057.2020

Vagus nerve/long-COVID

Conclusion:

Study suggests – non-invasive stimulation of the auricular branch of the vagus nerve of tVNS treatment is possible therapeutic modality for treating long-COVID

BH Natelson, M Blate, T Soto. **Transcutaneous Vagus Nerve Stimulation in the Treatment of Long Covid-Chronic Fatigue Syndrome**, *medRxiv* 2022.11.08.22281807

Chronic vagus nerve stimulation reduces body fat, blood cholesterol and triglyceride levels in rats

Conclusion:

Chronic electrical VNS exerts anorexigenic effects, lowering blood concentration of lipids

Gil K, Bugajski A, Kurnik M, Thor P. **Chronic vagus nerve stimulation reduces body fat, blood cholesterol and triglyceride levels in rats fed a high-fat diet**. *Folia Med Cracov*. 2012;52(3-4):79-96. PMID: 24852689

VNS in musculoskeletal diseases

Findings:

- In rheumatoid arthritis (RA) patients, VNS shown to dampen inflammatory response of circulatory peripheral cells
- Limit fatigue in Sjogren's syndrome and systemic lupus
- Decrease pain in fibromyalgia
- Decrease pain in erosive hand osteoarthritis

Courties A, Berenbaum F, Sellam J. **Vagus nerve stimulation in musculoskeletal diseases**. *Joint Bone Spine*. 2021 May;88(3):105149. Epub 2021 Feb 3

Vagus nerve stimulation

- Vagus nerve stimulation:
  - activates inflammatory reflex
  - neural reflex that modulates innate and adaptive immunity in response to pro inflammatory mediators
  - Safe/effective treatment for children/young adults with mild to moderate IBD
  - 50% of Crohn's disease, 43% ulcerative colitis – achieved clinical remission

Sahn B, et al. **Non-invasive vagal nerve stimulation to treat Crohn disease and ulcerative colitis in children and young adults.** *AIBD* 2021;poster #072

Vagus nerve stimulation(VNS)/stroke recovery

Human studies indicate brief bursts of VNS in conjunction with rehabilitative training improve recovery of motor function after stroke

- Doubles long-lasting recovery on complex task involving forelimb supination
- Doubles recovery on simple motor task not paired with VNS
- Enhances structural plasticity in motor networks

Teresa JK, David P, Cecilia NP, et al. **Vagus Nerve Stimulation Paired With Upper Limb Rehabilitation After Chronic Stroke.** *Stroke*, 27 Sept 2018;49:2789–2792

VNS inhibits cytokine production and attenuates disease severity in RA

Findings:

- VNS in RA patients significantly inhibited TNF production for up to 84 days
- RA disease severity as measured by standardized clinical composite scores improved significantly

Conclusion:

Vagus nerve stimulation targeting the inflammatory reflex modulates TNF production and reduces inflammation in humans

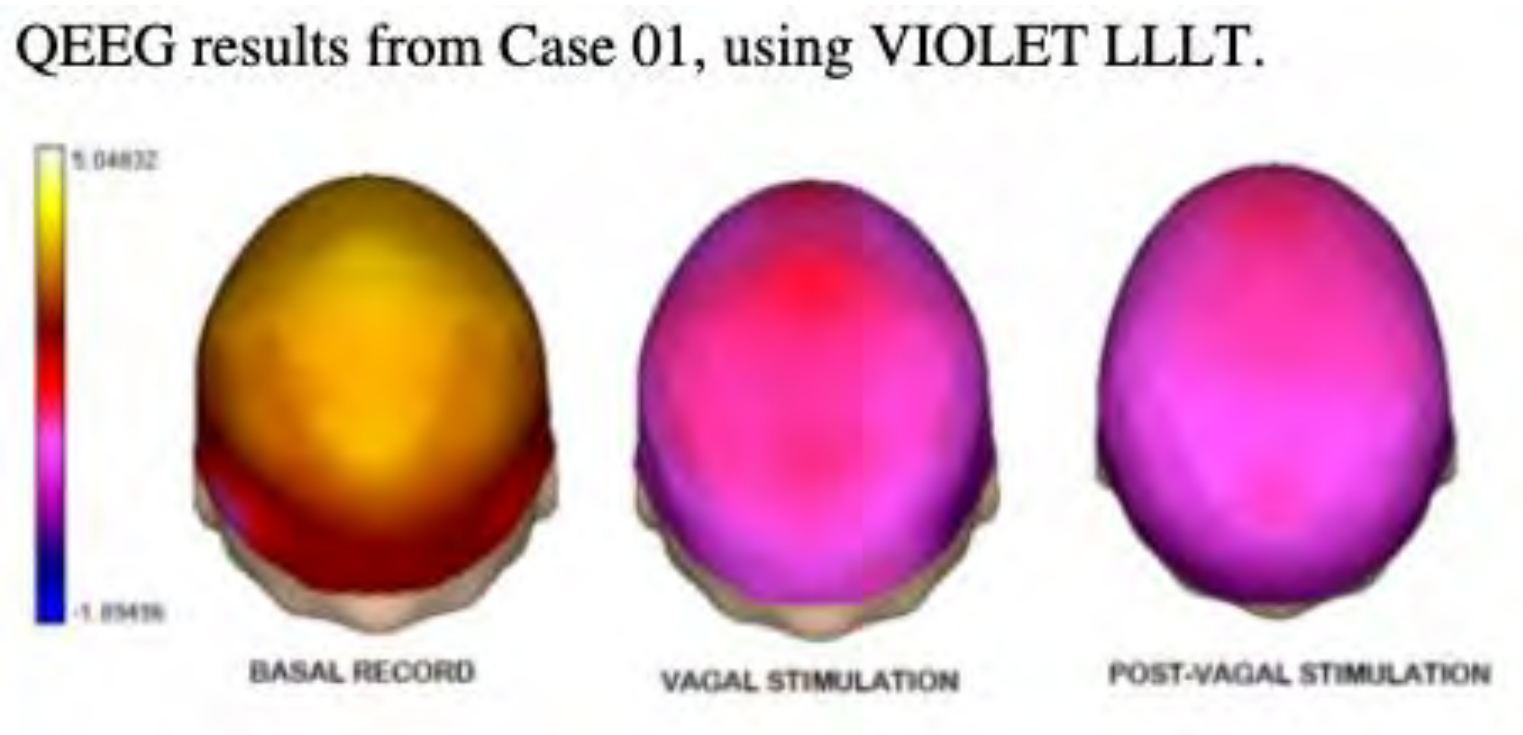
Koopman FA, Chavan SS, Miljko S, et al. **Vagus nerve stimulation inhibits cytokine production and attenuates disease severity in rheumatoid arthritis**. *Proc Natl Acad Sci U S A*. 5 July 2016 Jul 19;113(29):8284-9

Vagus nerve/Parkinson's

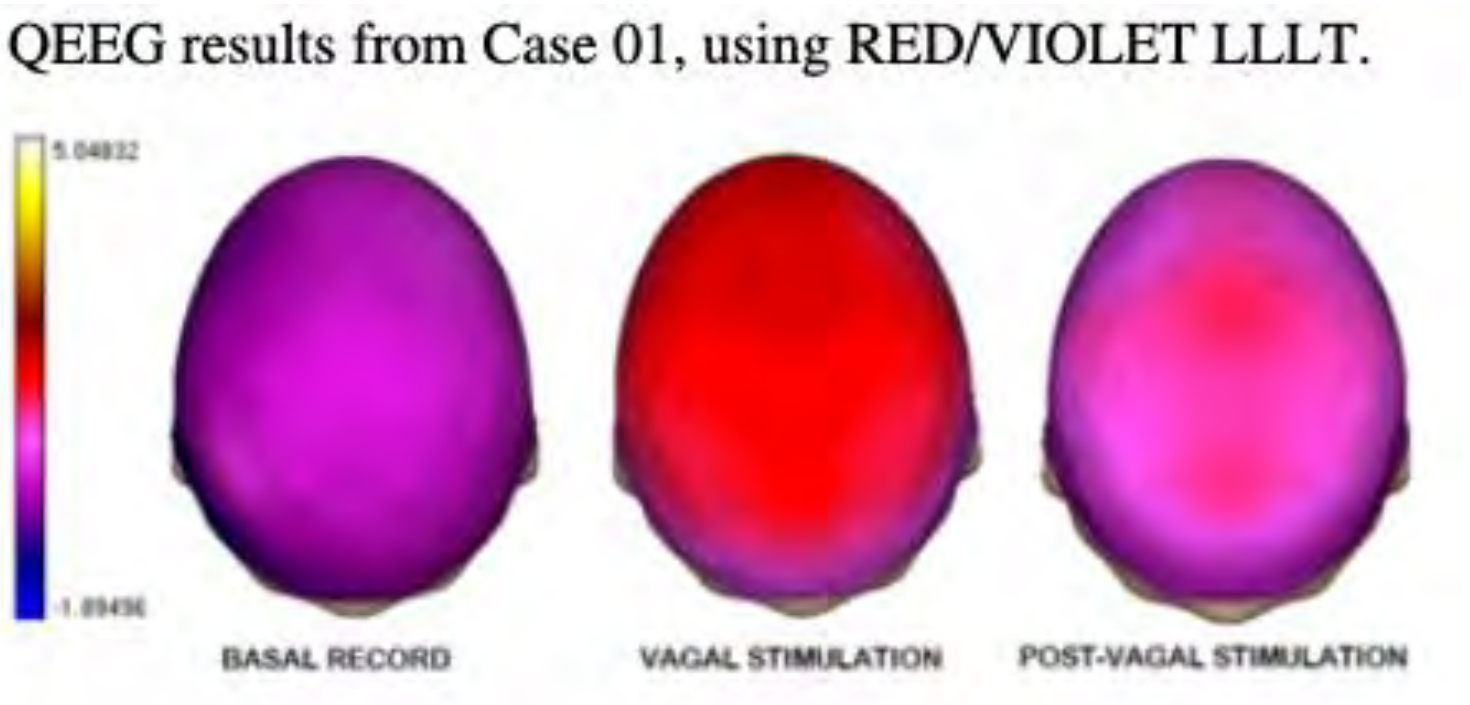
"The vagal nerve might provide a path for the spread of alpha-synuclein pathology from the ENS to the brain through the brainstem, midbrain, basal forebrain and finally the cortical areas".

Paula PP, Tessa K, Hemraj BD, et al. **The gut-brain axis in Parkinson's disease: Possibilities for food-based therapies**. *European Journal of Pharmacology*, 15 Dec 2017;817:86-95

Non-Thermal Vagal Nerve Stimulation



Machado, Calixto & Machado, Yanin & Chinchilla, Mauricio & Foyaca-Sibat, Humberto. (2019). Vagal Nerve Stimulation With Low Level Lasers Of Two Different Frequencies, Assessed By QEEG. Internet Journal of Neurology. 21. 1-9. 10.5580/IJN.54122.



Machado, Calixto & Machado, Yanin & Chinchilla, Mauricio & Foyaca-Sibat, Humberto. (2019). Vagal Nerve Stimulation With Low Level Lasers Of Two Different Frequencies, Assessed By QEEG. Internet Journal of Neurology. 21. 1-9. 10.5580/IJN.54122.

Vagal nerve stimulation with low-level lasers of 2 difference frequencies

Conclusion:

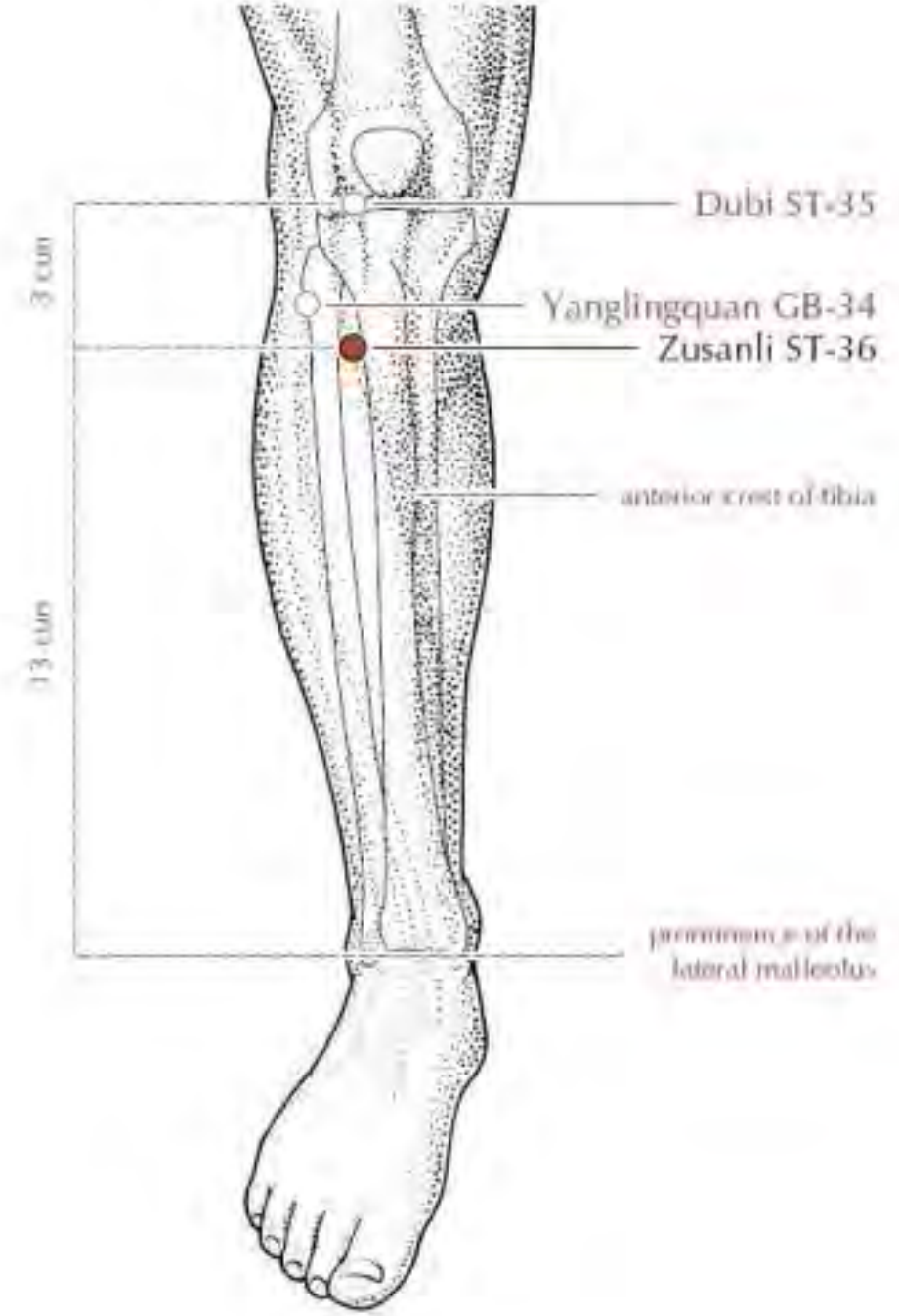
- Results using LLLT with violet light – effective in treatment of epilepsy
- Results with red/violet LLLT for VNS useful in conditions where necessary to induce increment of brain activity in conditions:
    - Depression
    - Neurorehabilitation
    - Coma
    - Disorders of consciousness
    - Dementia
    - Autism

C Machado, Y Machado, M Chinchilla, et al. **Vagal Nerve Stimulation With Low Level Lasers Of Two Different Frequencies**, Assessed By QEEG. *The Internet Journal of Neurology*. 2019;21:1

Vagus nerve demo

The perfect 10

Acupressure point St 36

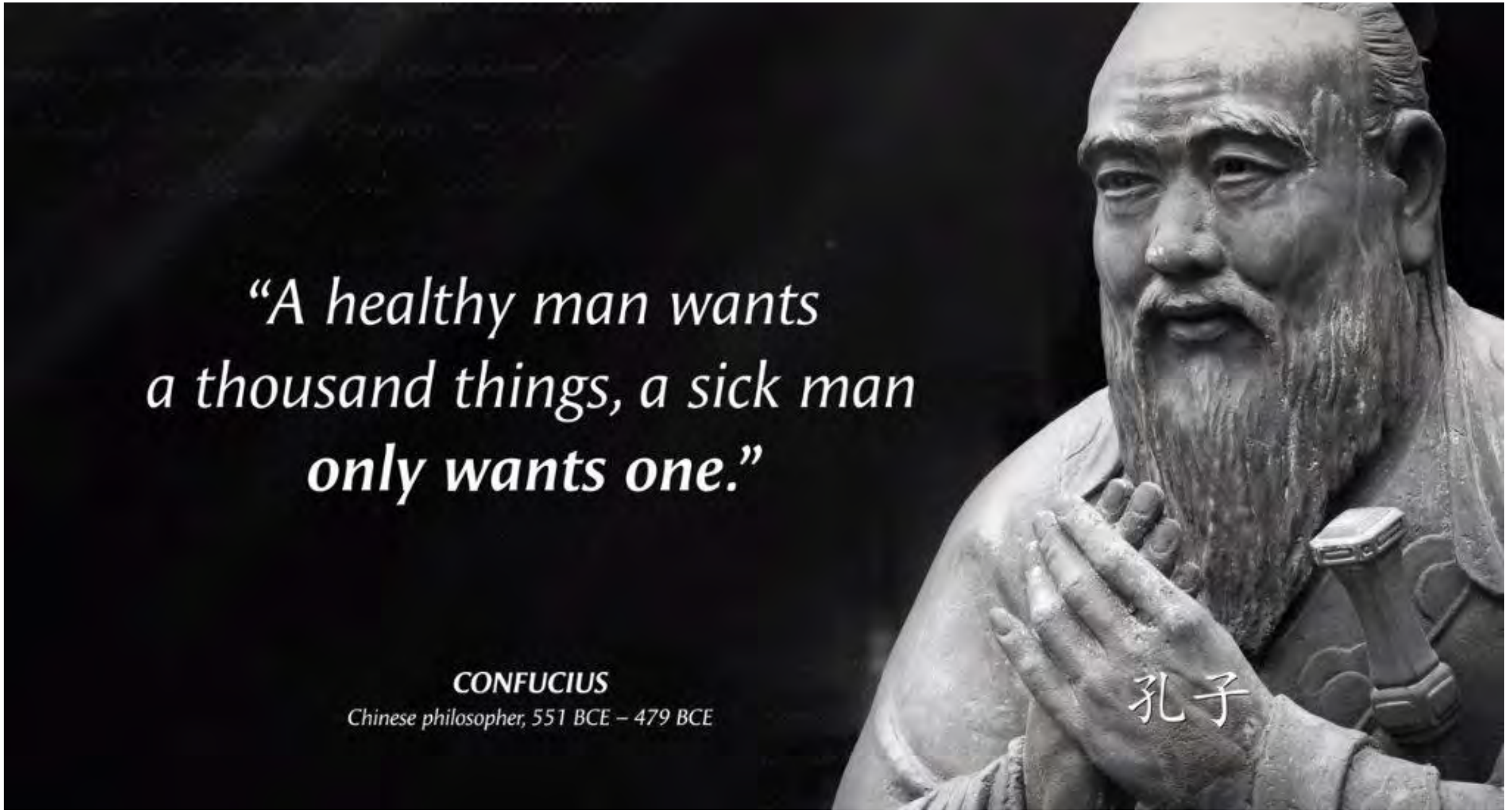


VAGUS NERVE NUTRITIONAL SUPPORT

Function	Product	Dosage
Vagally-mediated probiotics	**UltraBiotic Daily Multi-Strain**	1 cap daily
Short-chain fatty acids (butyric acid) - direct effect on afferent sensory firing	**Dynamic GI Integrity**	1 scp daily
Fiber – increases GLP-1	**Dynamic GI Integrity**	1 scp daily
Increase bile acid flow	**Digestive Complete**	2 caps daily
Omega-3 fatty acids – increases HRV	**Omega Pure EPA-DHA 1000**	2 sg BID
L-citrulline – increases HRV	**Pure L-Citrulline**	1 cap daily
Time-restricted eating (TRE)		
Sleep more	Liposomal Sleep	2 full droppers daily

Metabolism. 2012 Sept;61(9):1312-20. Epub 2012 Mar 24
Mol Metab. 2014 Sept;3(6):595-607. Epub 2014 Jun 27

Key Laser Therapy **Protocols** for **musculoskeletal Injuries**


"A healthy man wants
a thousand things, a sick man
only wants one."
CONFUCIUS
Chinese philosopher, 551 BCE – 479 BCE
孔子

Rotator Cuff (Impingement Syndrome): Causes

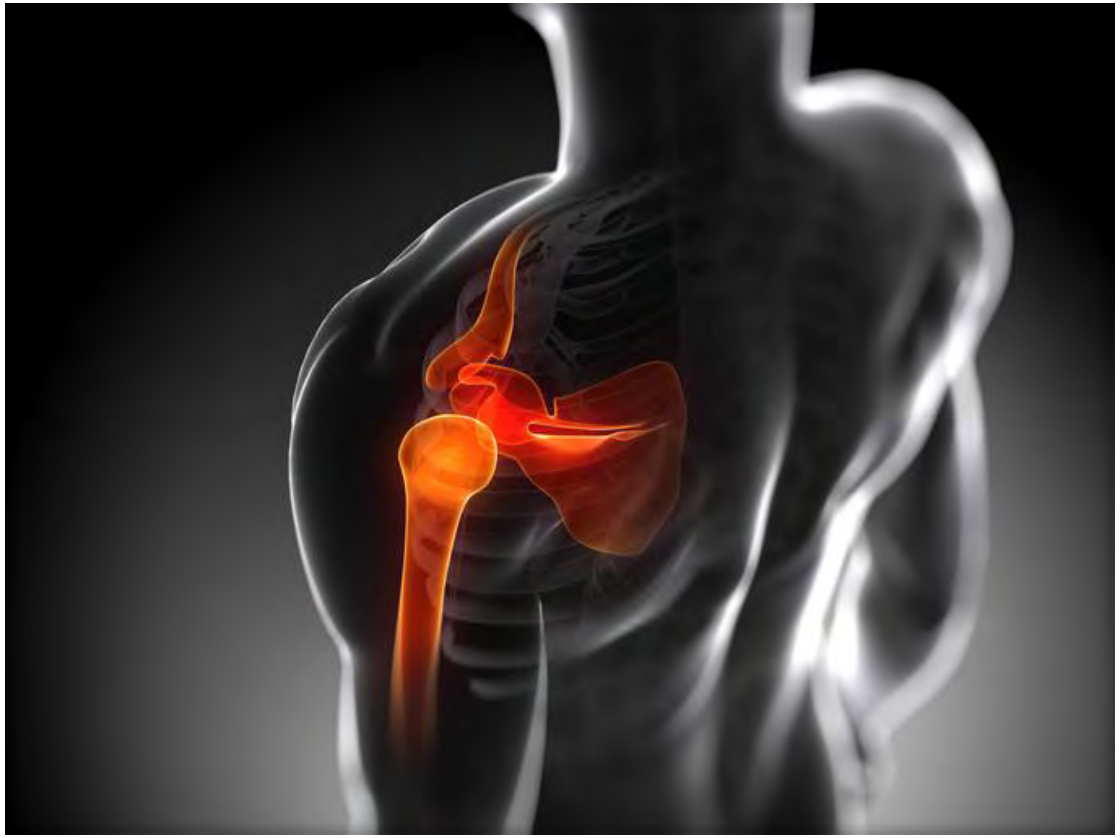
- Tendinopathy
- Wear and tear – collagen breakdown
- Poor posture
- Scapula orientation
- Falling – overstretch arm, bracing with arm
- Repetitive stress
- Heavy lifting activities

Risk Factors

- Age, being an athlete, posture, weak shoulder muscles

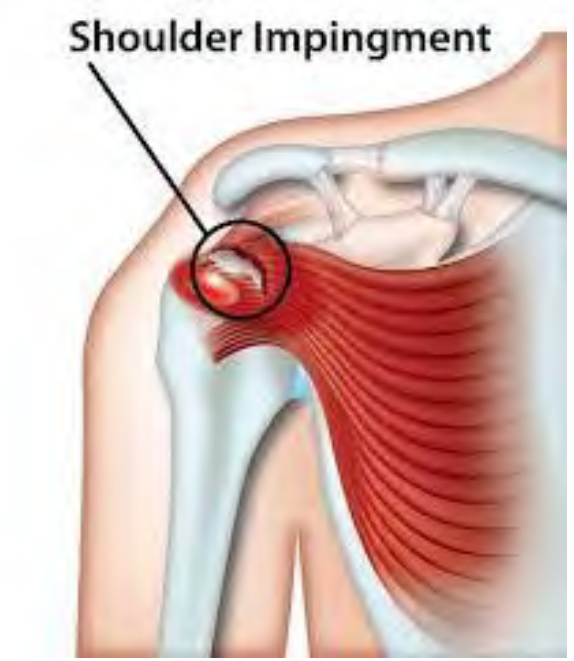
The shoulder dysfunction continuum

- Scapular dyskinesis
- Anterior impingement syndrome
- Rotator cuff tear
- Rotator cuff rupture



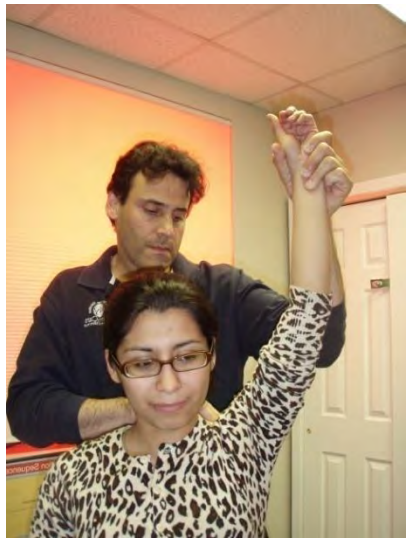
Shoulder impingement

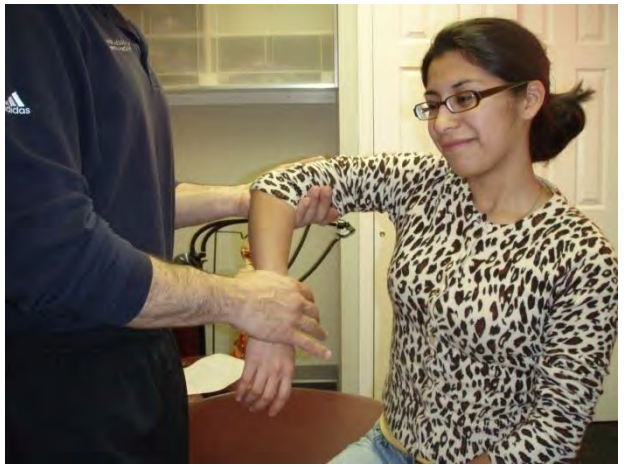
- Those with shoulder impingement had greater thoracic kyphosis and less extension ROM than healthy controls
- 35% of patients with shoulder anterior impingement syndrome had cervical nerve root compression on same side



Hunter DJ, Rivett DA, McKiernan S, et al. *Phys Ther*, 2019 Dec 11 online

Tests





- ROM
- Palpation of muscles
- Rotator cuff tear
- Subacromial impingement
- Labrum
- Shoulder cross-over test:
  - Scapular assistance test
  - Scapular retraction test
- D/D other shoulder pathologies and/or cervical post syndrome

Exercise and LLLT for Subacromial Impingement

- **Conclusion:** This double-blind, randomized control trial showed that LLT and exercise therapy is more effective than exercise therapy alone for the purposes of improving pain and active/passive ROM in patients with subacromial syndrome

Clinical Rheumatology 2011; 30: p1341-46

Frozen Shoulder: The Effectiveness of Conservative and Surgical Interventions

Conclusion: Strong evidence for the effectiveness of laser therapy

British Journal of Medicine 2011, Jan. 45(1), p.49-58

LLLT treatment in patients with frozen shoulder

Results: Laser vs. placebo group

- Significant decrease in overall, night, and activity pain scores at end of 4, 8, 16 weeks
- Significant decrease in shoulder pain and disability index (SPADI) scores end of 4, 8, 16 weeks
- Significant decrease in disability of arm, shoulder, and hand questionnaire (DASH) scores at the end of 8 and 16 weeks
- Significant decrease in HAQ scores at end of 4 & 8 weeks

Stergioulas A. **Low-power laser treatment in patients with frozen shoulder: preliminary results**. *Photomed Laser Surg*. 2008 Apr;26(2):99-105

Efficacy of LLLT for shoulder tendinopathy

Conclusion:

- 17 randomized controlled trials (RCTs)
- Optimal LLLT can offer clinically relevant pain relief and initiate a more rapid course of improvement, both alone and in combination with physiotherapy interventions

Haslerud S, Magnussen LH, Joensen J, et al. **The efficacy of low-level laser therapy for shoulder tendinopathy: a systematic review and meta-analysis of randomized controlled trials**. *Physiother Res Int*. 2015 Jun;20(2):108-25

LLLT combined with exercise for subacromial impingement

Conclusion:

LLLT combined with exercises:

- Reduce pain intensity
- Improve shoulder function
- reduces medication intake over 3 months

Alfredo PP, Bjordal JM, Junior WS, et al. **Efficacy of low-level laser therapy combined with exercise for subacromial impingement syndrome: A randomised controlled trial.** *Clin Rehabil*. 2021 Jun;35(6):851-860

Rotator Cuff Injury Treatment Protocol

- Laser at point/points of involvement:
  - Muscle/joint/scapular (9,16,42,53)
- Laser during movement – 30-60 sec.
- Laser “locomotor lock-in”
- Laser “core lock-in”
- Corrective exercise
- Mobilize, manipulate joint restrictions
- Myofascial release

Shoulder tension release

- Laser scapula – head 1
- Ossillate the arm
- Turn arm towards ER at 90^{o} of abduction
- Laser shoulder with other head (2)

Exercise Recommendations Rotator Cuff

Sleeper Stretch

Rotator Cuff External Rotation

Cross Body Stretch

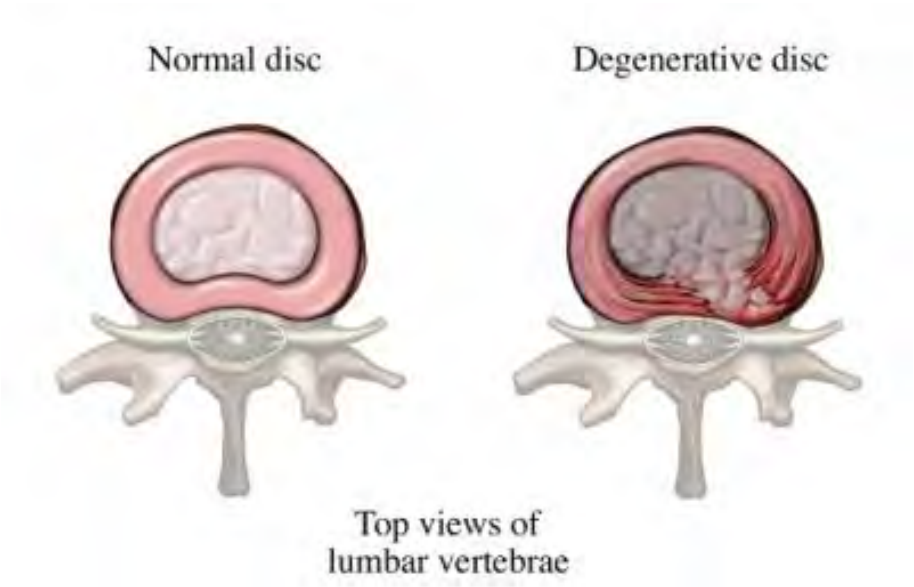
Rotator Cuff Internal Rotation

Scapular Protraction

Seated High Rows

Intervertebral Disc Disease

- IVD – the largest structure in the body without vascular supply



Top views of
lumbar vertebrae

Intervertebral Disc Disease (cont'd)

- Nucleus – gel-like substance
  - Water (80%)
  - Type II collagen fibers (17%)
  - Proteoglycans (PG) (65%)
  - Small amount of elastin fibers
- Annulus – 65% water:
  - Outer layer (thinner): made up of more disorganized collagen bundles and a greater proportion of vertical fibers (almost all type-1 collagen)
  - Inner layer: made up of more water, PG, and predominantly type-II collagen

Intervertebral Disc Disease (cont'd)

- IVD deteriorate over years (from nucleus outward)
- Influenced by:
  - Genetic inheritance
  - Metabolite transport
  - Age-related deterioration can be accelerated by physical disruption
- Degeneration most often occurs in lower lumbar discs

Healing of disc periphery has potential to relieve discogenic pain by re-establishing a physical barrier between nucleus and nerves, and reducing inflammation.

Diagnostic Accuracy of the Slump Test for Identifying Neuropathic Pain (NP) in the Lower Limb

- Slump test displayed high sensitivity within study sample of individuals with LBP
- Conversely, adding criterion of pain distal to knee during slump test yielded very high specificity

JOSPT, Aug 2015;45(8):596-603

Intervertebral Disc Tx Protocol

- Laser at point/point of involvement
- Laser during movement: 30-60 sec.
- Laser “locomotor lock-in”
- Corrective exercise:
  - Bracing
  - McGill big 4
  - Hip flexor stretched
  - KB swings

Intervertebral Disc Treatment Protocol (cont'd)

- Core "lock-in"
- Nutritional protocol
- Myofascial release
- Mobilize, manipulate, flex/distraction, joint restrictions

Pro-inflammatory biomarkers and low-back pain

Conclusion:

- Positive association between CRP and IL-6 and severity of NsLBP
- Positive association between TNF-Alpha and presence of NsLBP

Van den Berg R, Jongbloed EM, de Schepper EIT, et al. **The association between pro-inflammatory biomarkers and nonspecific low back pain: a systematic review**. *Spine J*, 2018 Jun 27

Interleukin-1β in intervertebral disk degeneration

- Interleukin-1 (IL-1) β has strong pro-inflammatory activity by stimulating the secretion of multiple pro-inflammatory mediators
- IL-1β is highly expressed in degenerative intervertebral disk (IVD) tissues and cells
- Inhibition of IL-1β found to promote extracellular matrix (ECM) repair and protect against disk degeneration

Yang W, et al. *Clin Chim Acta* 2015

New approach to diagnosing low back pain

- Findings determined serum levels of IL-6 significantly higher in subjects with low back pain compared with control participants
- Participants with low back pain due to spinal stenosis or degenerative disc disease also had higher levels than those with intervertebral disc herniation and controls
- Findings suggest that patients with low back pain have low-grade systemic inflammation
- Biochemical profiling or circulating cytokines can assist in diagnosing those with low back pain

North Shore-Long Island Jewish (LIJ) Health System-Jan-7-2016

LLLT – spinal cord injury

- LLLT allowed neurons to survive
- LLLT elevated IL-4 and IL-13
- Results show that LLLT:
  - Has potential for reducing inflammation
  - Regulates macrophage/microglial
  - Promoting neuronal survival
- LLLT may be an effective candidate for treatment of spinal cord injury

LLLT facilitates alternatively activated macrophage. *Nature.com*, Apr 4, 2017

Effectiveness of LLLT in patients with discogenic lumbar radiculopathy

- 110 patients
- 55 patients treated with LLLT and conventional PT
- 55 patients treated with conventional PT alone
- Both groups received 18 treatment sessions

Results: LLLT/PT group had significant improvements over PT alone:

- Local trunk movements
- Pain intensity
- Related functional disability
- No side-effects after LLLT use

Ahmed I, Bandpei MAM, Gilani SA, et al. **Effectiveness of Low-Level Laser Therapy in Patients with Discogenic Lumbar Radiculopathy: A Double-Blind Randomized Controlled Trial**. *J Healthc Eng*. 2022 Feb 27;2022:6437523

LLLT on pain relief and interleukin-6

Conclusion:

Long periods of LLLT have better effects in improving complication of spinal cord injury (SCI). Since LLLT does not cause the side effects of MPSS, long term use of LLLT may be proper alternative for MPSS in decreasing post SCI side effects

Mojarad N, Janzadeh A, et al. *J Chem Neuroanat*, 2018 Jan;87:60-70

Low level light therapy (LLLT) modulates inflammatory mediators secreted by human annulus fibrosus (AF) cells during intervertebral disc degeneration in vitro

Key Takeaway:

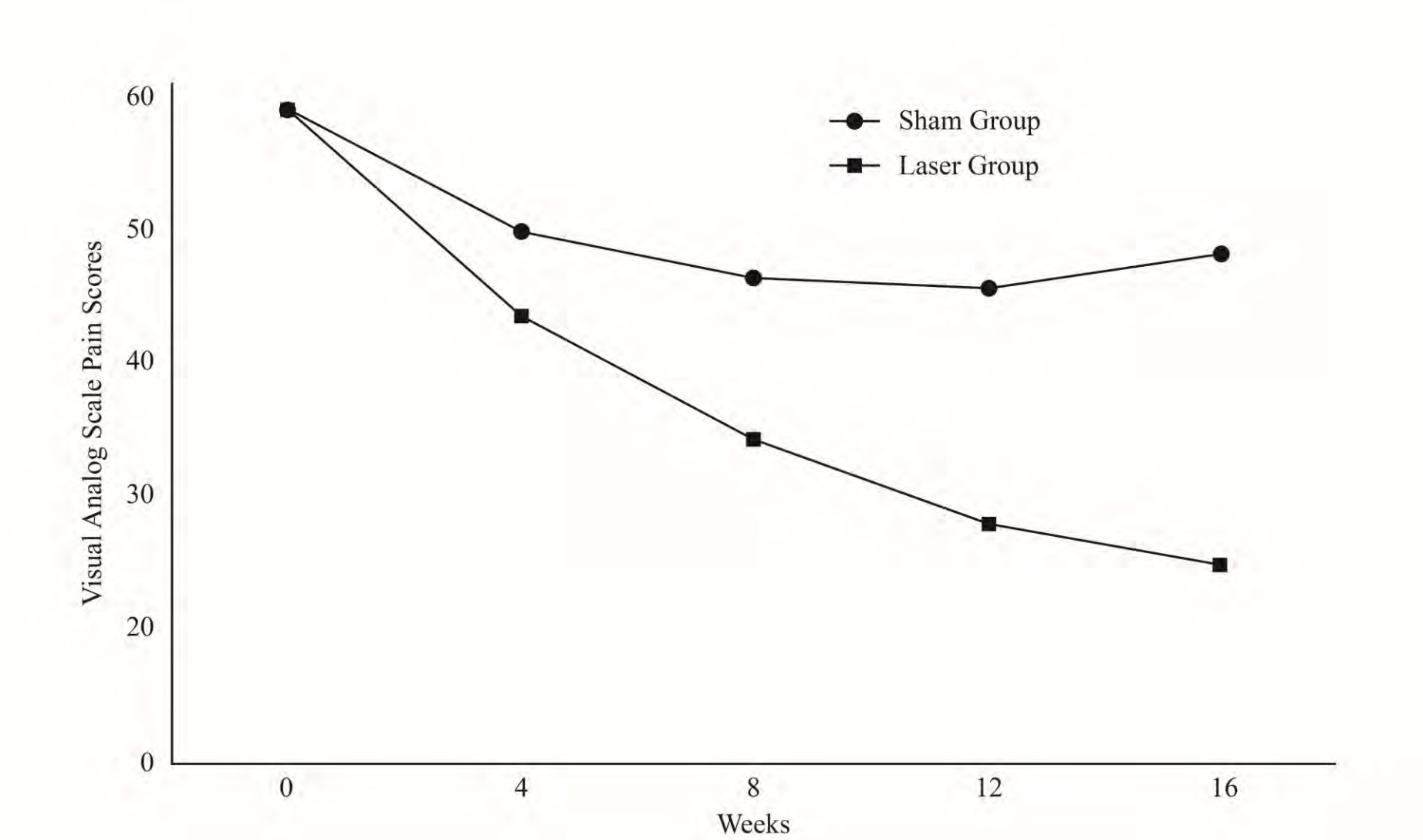
- Inflammatory microenvironment in AF cells suppressed by LLLT (IL-6 and 8 levels)
- Results indicate LLLT is potential method of IVD treatment
- 405 NM – most positively affected IL-6

Photochem Photobiol 2015, Mar-Apr;91(2):403-10

LLLT for chronic low-back pain

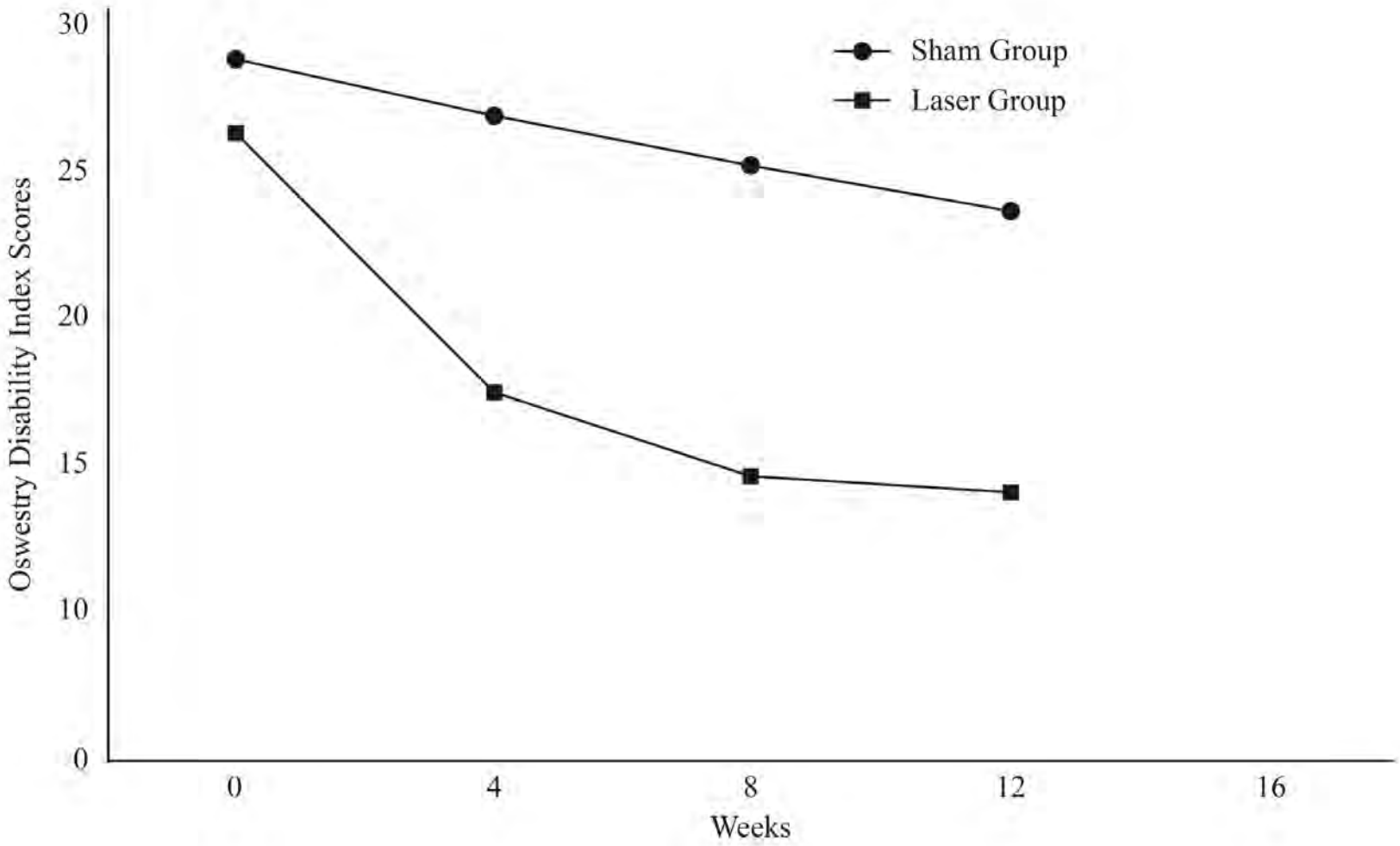
Lower Back - FX635

Changes in visual analog scale low-back pain scores



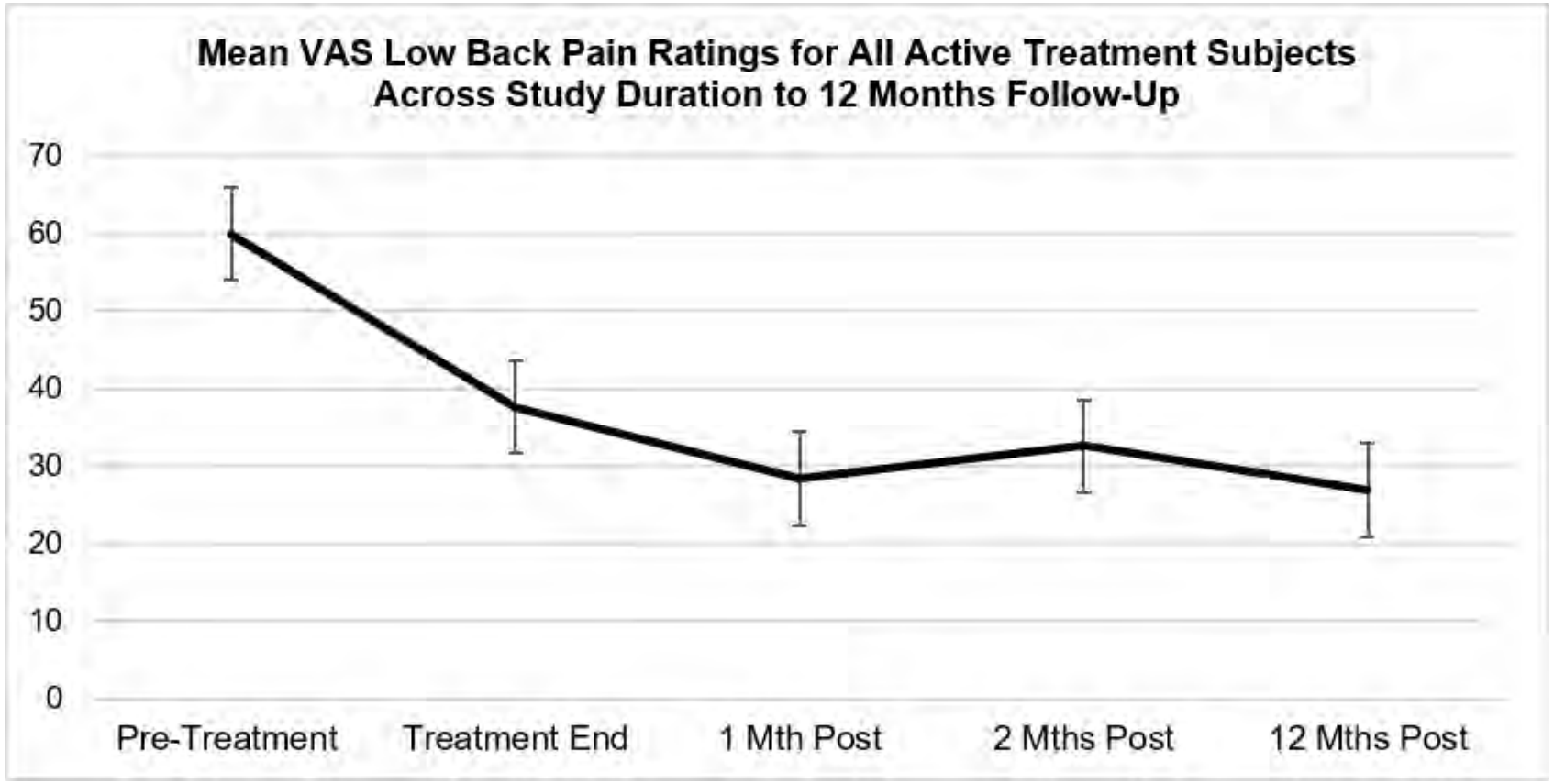
TS Berry, PJ Quarneri, G. Roche, TM Sammons. A Randomized, Double-blind, Sham-Controlled Study Evaluating the Effectiveness of a Low-level laser Device for Treating Lower-Back Pain**.** *eMedical Res. 2:*10005

Changes in Oswestry disability index scores

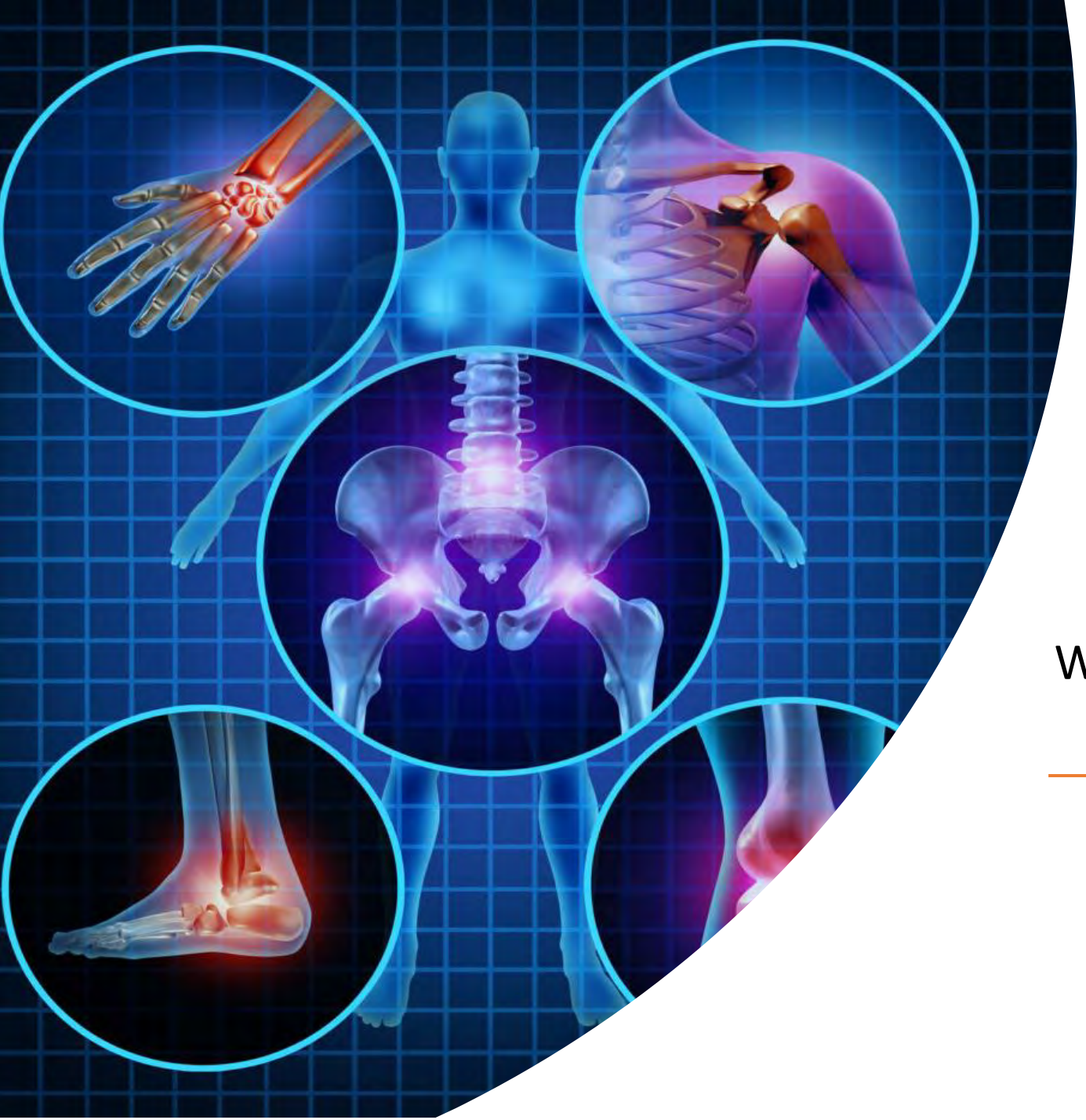


TS Berry, PJ Quarneri, G. Roche, TM Sammons. A Randomized, Double-blind, Sham-Controlled Study Evaluating the Effectiveness of a Low-level laser Device for Treating Lower-Back Pain**.** *eMedical Res. 2:*10005

LLLT for treating LBP: 12-month follow-up



TS Berry, PJ Quarneri, G. Roche, TM Sammons. **Low-Level Laser Therapy for Treating Low-Back Pain.** *J. Pain Relief*, 2019;9(1):1000347



October 2021

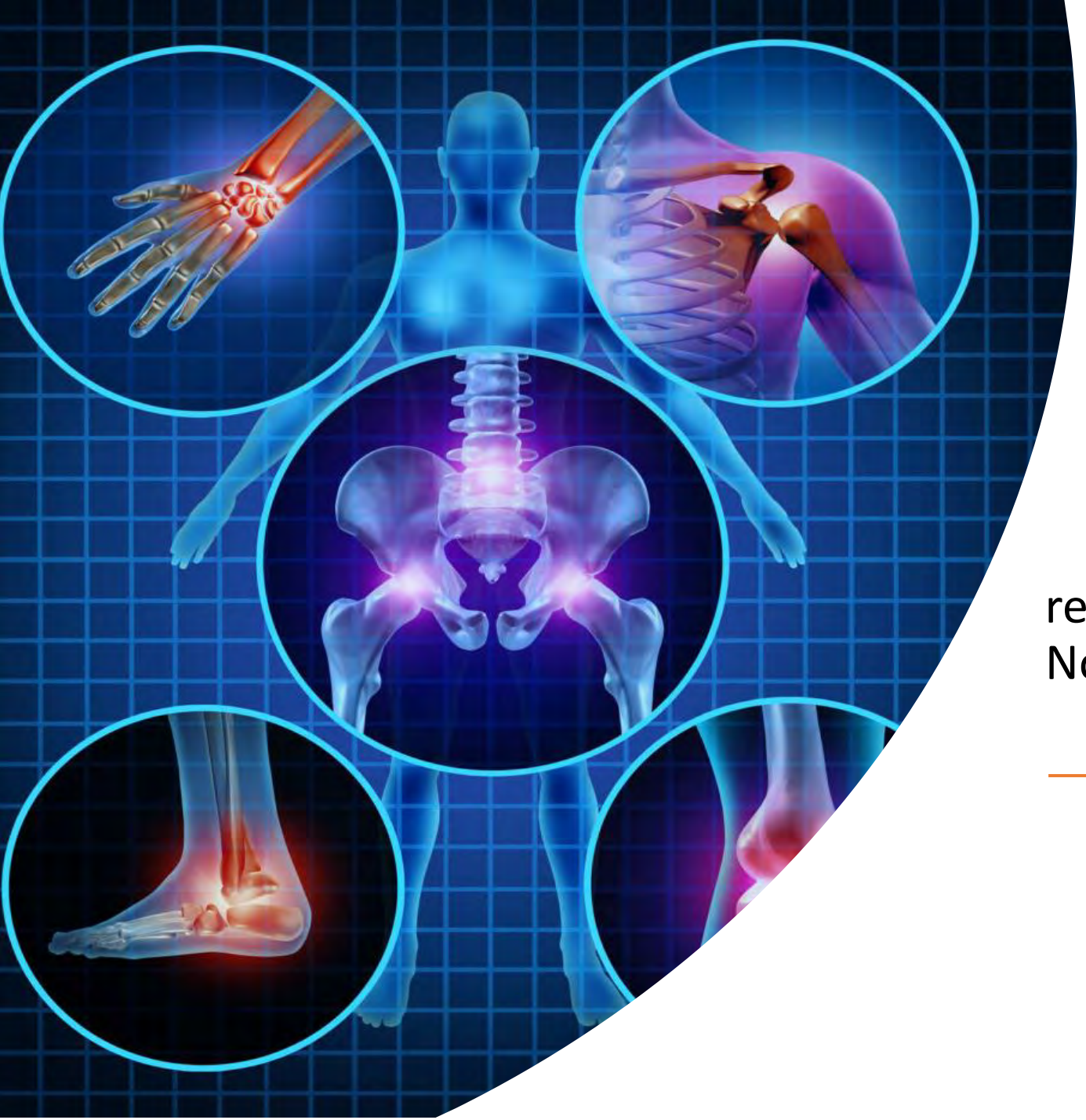
Whole Body Post-Operative Pain

#19
FX635

FX635

Whole Body Treatment of Postoperative Pain

- Double-blind and placebo
- 255 patients
- 49% average pain reduction post-treatment protocols
- No other therapies used
- Comparison (JAMA 2018) – 119 patients:
  - Opioids – 20%
  - Non-opioids – 26% (mainly NSAIDs)
- Implemented adjunctive therapies



November 2021

1st and Only

Noninvasive Technology to receive FDA Clearance for Overall Nociceptive Musculoskeletal Pain

#20 FX405

FX405 – the world's MOST ADVANCED laser

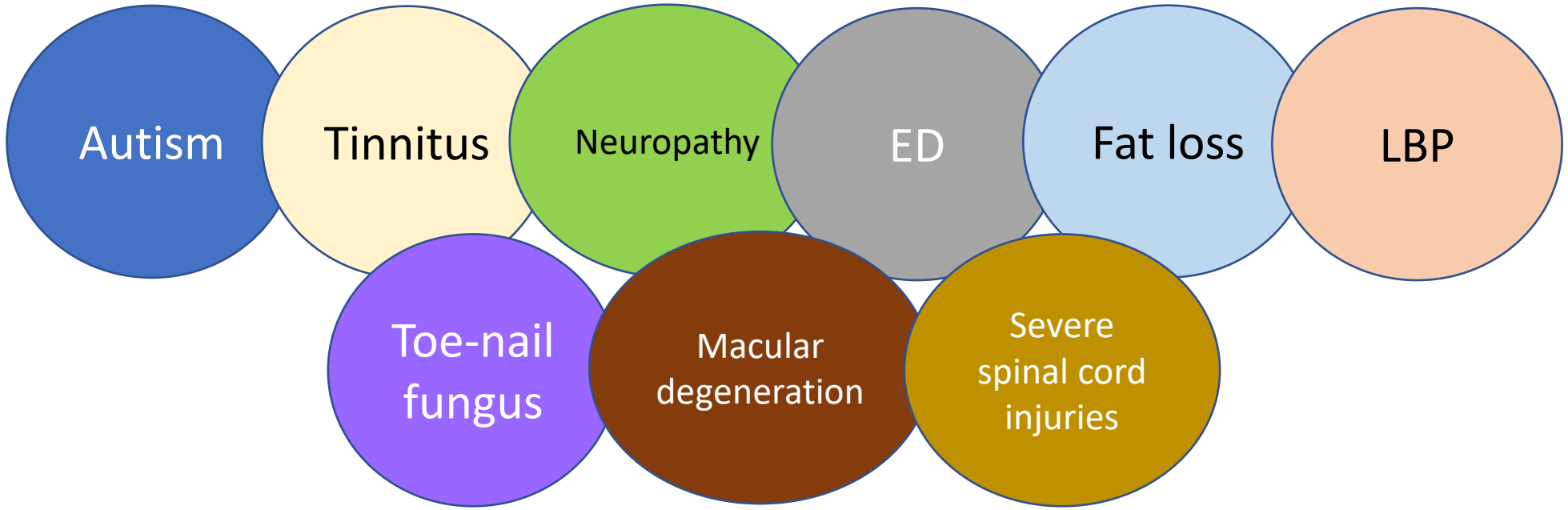
- 3 red diodes – 17.5 mw
- 1 violet diode (new) – 23 mw

Dr. Rob's take:

- 20% improvement in outcome
- In half the time

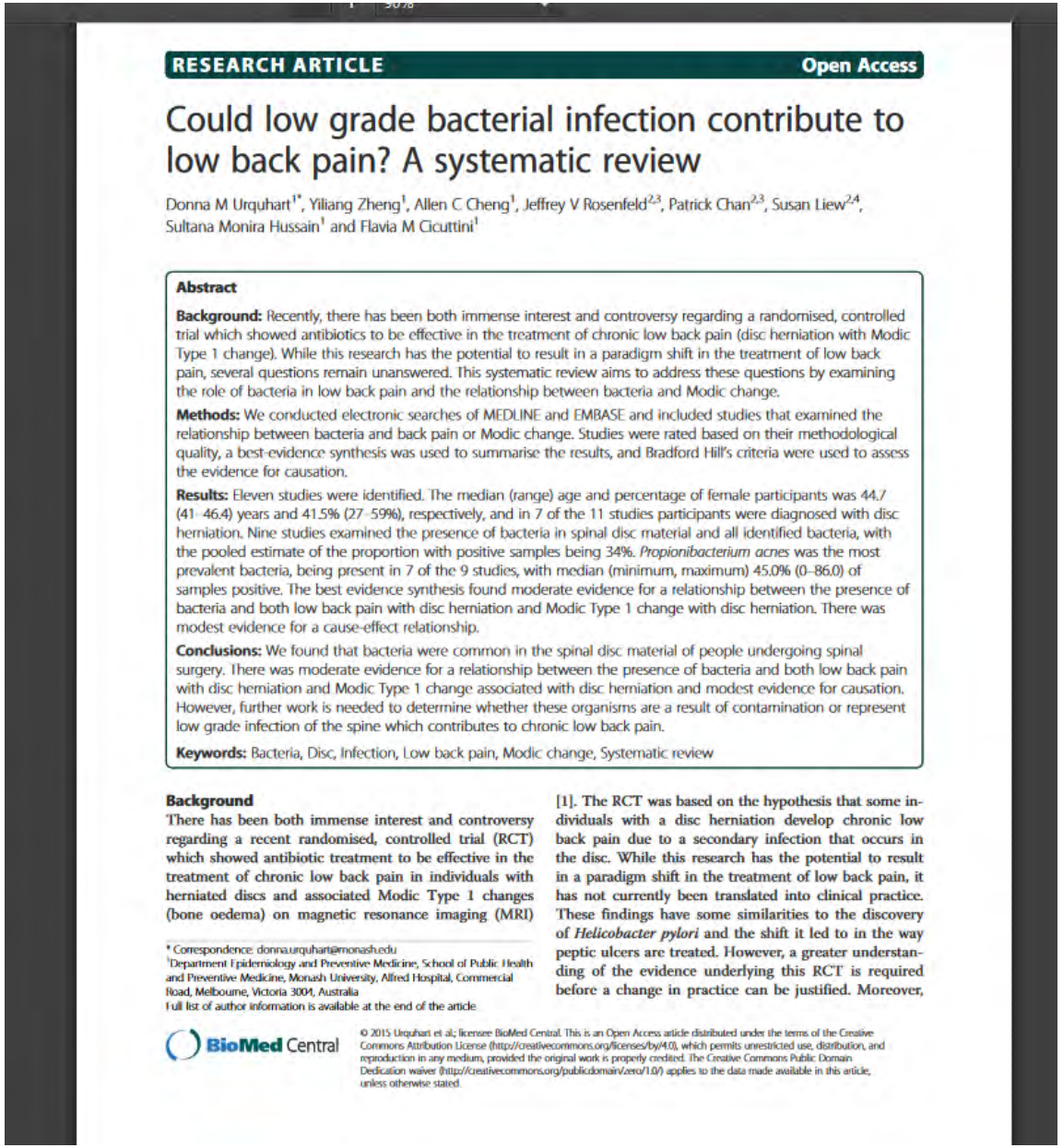
Erchonia

Current research/projects/collabs



Testing a theory

Laser using FX635 and Violet to Treat Low Back Pain



RESEARCH ARTICLE Open Access

Could low grade bacterial infection contribute to low back pain? A systematic review

Donna M Urquhart[1*], Yiliang Zheng[1], Allen C Cheng[1], Jeffrey V Rosenfeld[2,3], Patrick Chan[2,3], Susan Liew[2,4], Sultana Monira Hussain[1] and Flavia M Cicuttini[1]

Abstract

Background: Recently, there has been both immense interest and controversy regarding a randomised, controlled trial which showed antibiotics to be effective in the treatment of chronic low back pain (disc herniation with Modic Type 1 change). While this research has the potential to result in a paradigm shift in the treatment of low back pain, several questions remain unanswered. This systematic review aims to address these questions by examining the role of bacteria in low back pain and the relationship between bacteria and Modic change.

Methods: We conducted electronic searches of MEDLINE and EMBASE and included studies that examined the relationship between bacteria and back pain or Modic change. Studies were rated based on their methodological quality, a best-evidence synthesis was used to summarise the results, and Bradford Hill's criteria were used to assess the evidence for causation.

Results: Eleven studies were identified. The median (range) age and percentage of female participants was 44.7 (41–46.4) years and 41.5% (27–59%), respectively, and in 7 of the 11 studies participants were diagnosed with disc herniation. Nine studies examined the presence of bacteria in spinal disc material and all identified bacteria, with the pooled estimate of the proportion with positive samples being 34%. *Propionibacterium acnes* was the most prevalent bacteria, being present in 7 of the 9 studies, with median (minimum, maximum) 45.0% (0–86.0) of samples positive. The best evidence synthesis found moderate evidence for a relationship between the presence of bacteria and both low back pain with disc herniation and Modic Type 1 change with disc herniation. There was modest evidence for a cause-effect relationship.

Conclusions: We found that bacteria were common in the spinal disc material of people undergoing spinal surgery. There was moderate evidence for a relationship between the presence of bacteria and both low back pain with disc herniation and Modic Type 1 change associated with disc herniation and modest evidence for causation. However, further work is needed to determine whether these organisms are a result of contamination or represent low grade infection of the spine which contributes to chronic low back pain.

Keywords: Bacteria, Disc, Infection, Low back pain, Modic change, Systematic review

Background

There has been both immense interest and controversy regarding a recent randomised, controlled trial (RCT) which showed antibiotic treatment to be effective in the treatment of chronic low back pain in individuals with herniated discs and associated Modic Type 1 changes (bone oedema) on magnetic resonance imaging (MRI) [1]. The RCT was based on the hypothesis that some individuals with a disc herniation develop chronic low back pain due to a secondary infection that occurs in the disc. While this research has the potential to result in a paradigm shift in the treatment of low back pain, it has not currently been translated into clinical practice. These findings have some similarities to the discovery of *Helicobacter pylori* and the shift it led to in the way peptic ulcers are treated. However, a greater understanding of the evidence underlying this RCT is required before a change in practice can be justified. Moreover,

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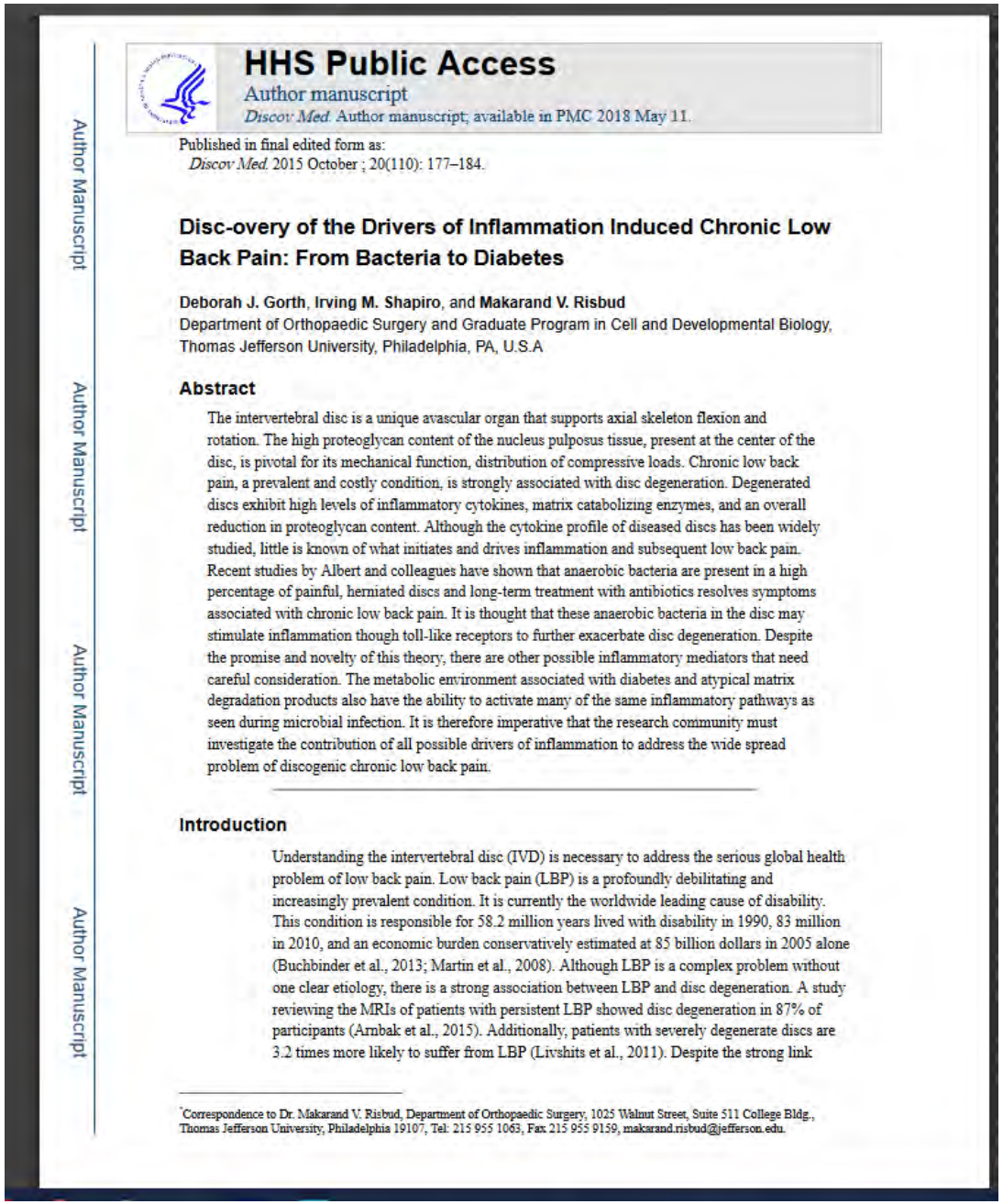
Conclusion:

- Bacteria common in spinal disc material of people undergoing spinal surgery
- Moderate evidence for relationship between presence of bacteria and both low back pain with disc herniation and Modic Type 1 change associated with disc herniation
- Modest evidence for causation

Urquhart, D.M., Zheng, Y., Cheng, A.C. *et al.* Could low grade bacterial infection contribute to low back pain? A systematic review. *BMC Med* **13,** 13 (2015)



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Discov Med. 2015 October ; 20(110): 177–184.

Disc-overy of the Drivers of Inflammation Induced Chronic Low Back Pain: From Bacteria to Diabetes

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Department of Orthopaedic Surgery and Graduate Program in Cell and Developmental Biology, Thomas Jefferson University, Philadelphia, PA, U.S.A

Abstract

The intervertebral disc is a unique avascular organ that supports axial skeleton flexion and rotation. The high proteoglycan content of the nucleus pulposus tissue, present at the center of the disc, is pivotal for its mechanical function, distribution of compressive loads. Chronic low back pain, a prevalent and costly condition, is strongly associated with disc degeneration. Degenerated discs exhibit high levels of inflammatory cytokines, matrix catabolizing enzymes, and an overall reduction in proteoglycan content. Although the cytokine profile of diseased discs has been widely studied, little is known of what initiates and drives inflammation and subsequent low back pain. Recent studies by Albert and colleagues have shown that anaerobic bacteria are present in a high percentage of painful, herniated discs and long-term treatment with antibiotics resolves symptoms associated with chronic low back pain. It is thought that these anaerobic bacteria in the disc may stimulate inflammation though toll-like receptors to further exacerbate disc degeneration. Despite the promise and novelty of this theory, there are other possible inflammatory mediators that need careful consideration. The metabolic environment associated with diabetes and atypical matrix degradation products also have the ability to activate many of the same inflammatory pathways as seen during microbial infection. It is therefore imperative that the research community must investigate the contribution of all possible drivers of inflammation to address the wide spread problem of discogenic chronic low back pain.

Introduction

Understanding the intervertebral disc (IVD) is necessary to address the serious global health problem of low back pain. Low back pain (LBP) is a profoundly debilitating and increasingly prevalent condition. It is currently the worldwide leading cause of disability. This condition is responsible for 58.2 million years lived with disability in 1990, 83 million in 2010, and an economic burden conservatively estimated at 85 billion dollars in 2005 alone (Buchbinder et al., 2013; Martin et al., 2008). Although LBP is a complex problem without one clear etiology, there is a strong association between LBP and disc degeneration. A study reviewing the MRIs of patients with persistent LBP showed disc degeneration in 87% of participants (Arnbak et al., 2015). Additionally, patients with severely degenerate discs are 3.2 times more likely to suffer from LBP (Livshits et al., 2011). Despite the strong link

*Correspondence to Dr. Makarand V. Risbud, Department of Orthopaedic Surgery, 1025 Walnut Street, Suite 511 College Bldg., Thomas Jefferson University, Philadelphia 19107, Tel: 215 955 1063, Fax 215 955 9159, makarand.risbud@jefferson.edu.

Conclusion:

- Data suggests anaerobic p. acnes could induce inflammatory effects on intervertebral disc through TLR signaling

- Understanding contribution and interplay between all inflammatory drivers - responsible and effective treatment for low back pain

Gorth DJ, Shapiro IM, Risbud MV. **Discovery of the drivers of inflammation induced chronic low back pain: from bacteria to diabetes**. *Discov Med.* 2015 Oct;20(110):177-84

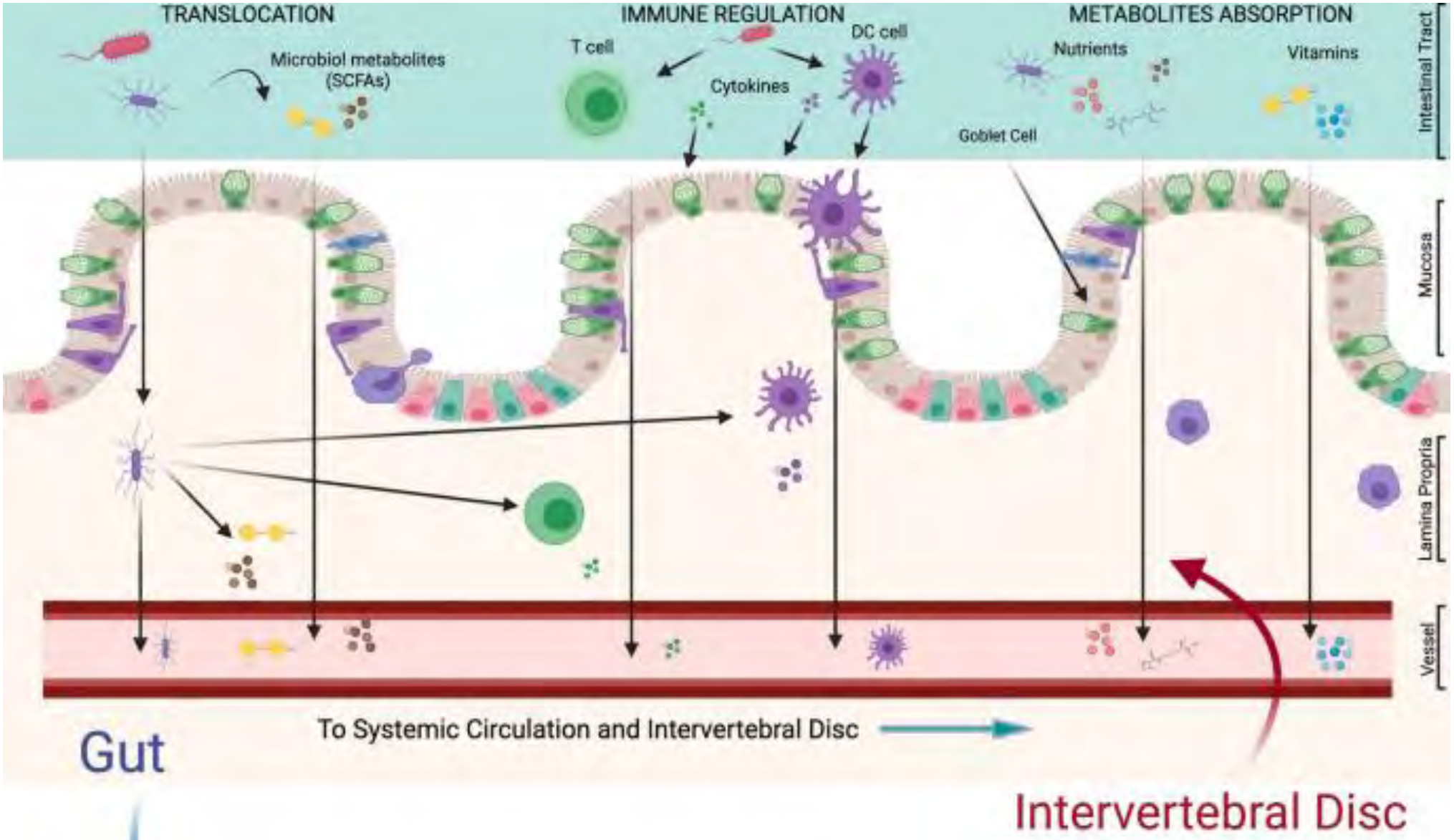
Is infection the possible initiator of disc disease?

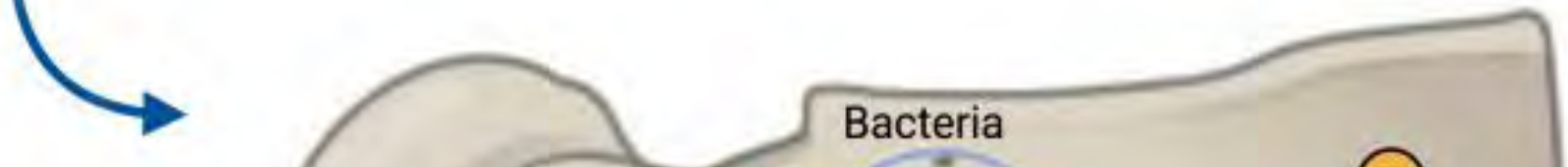
Conclusion:

Study demonstrates bacterial specific proteins and host defense proteins to infection which strengthen hypothesis of infection as possible initiator of disc disease

S Rajasekaran, C Tangavel, SN Aiyer, *et al.* ISSLS PRIZE IN CLINICAL SCIENCE 2017: **Is infection the possible initiator of disc disease? An insight from proteomic analysis**. *Eur Spine J*, 6 Feb 2021;26:1384–1400

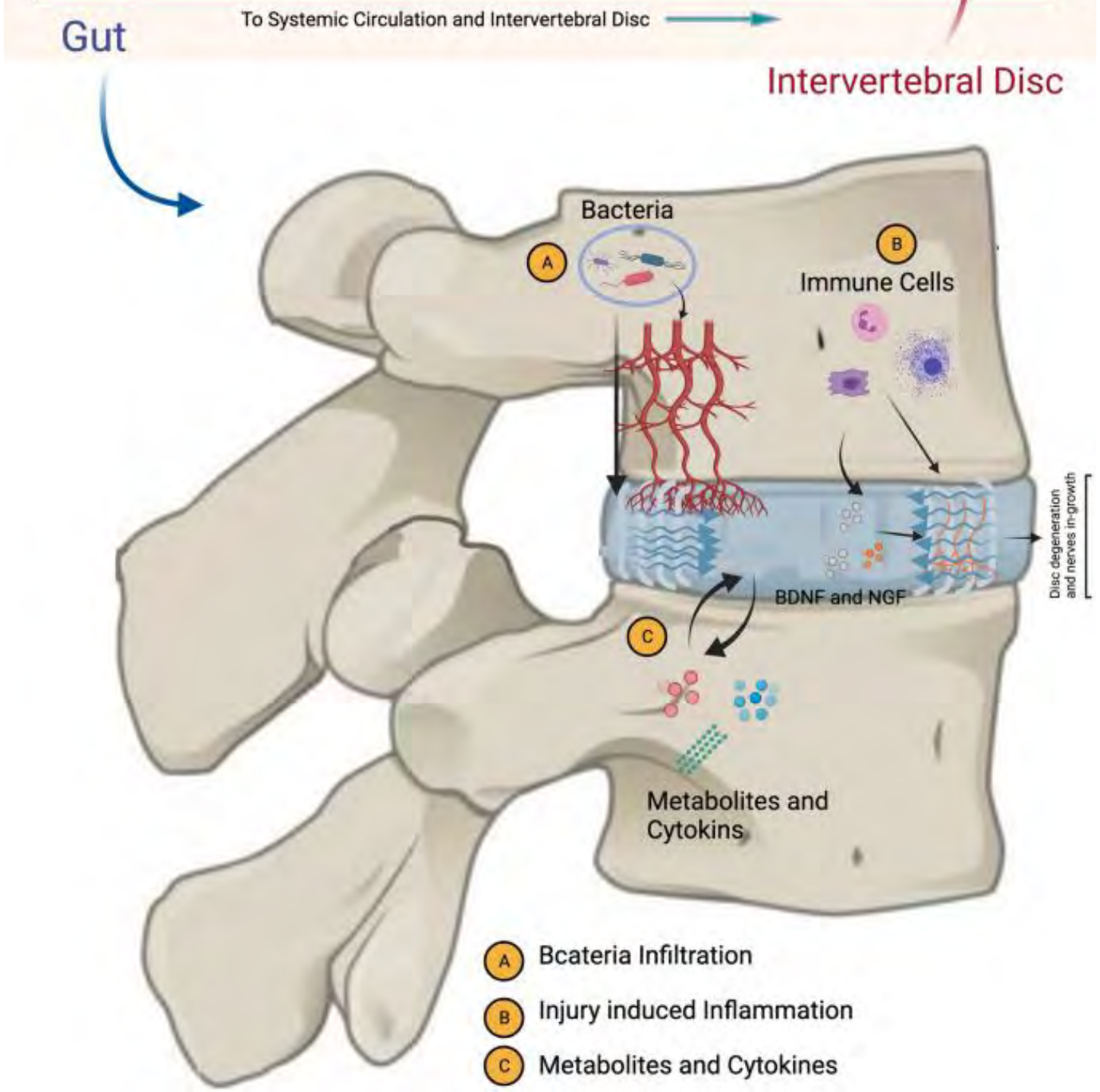
Gut-disc axis





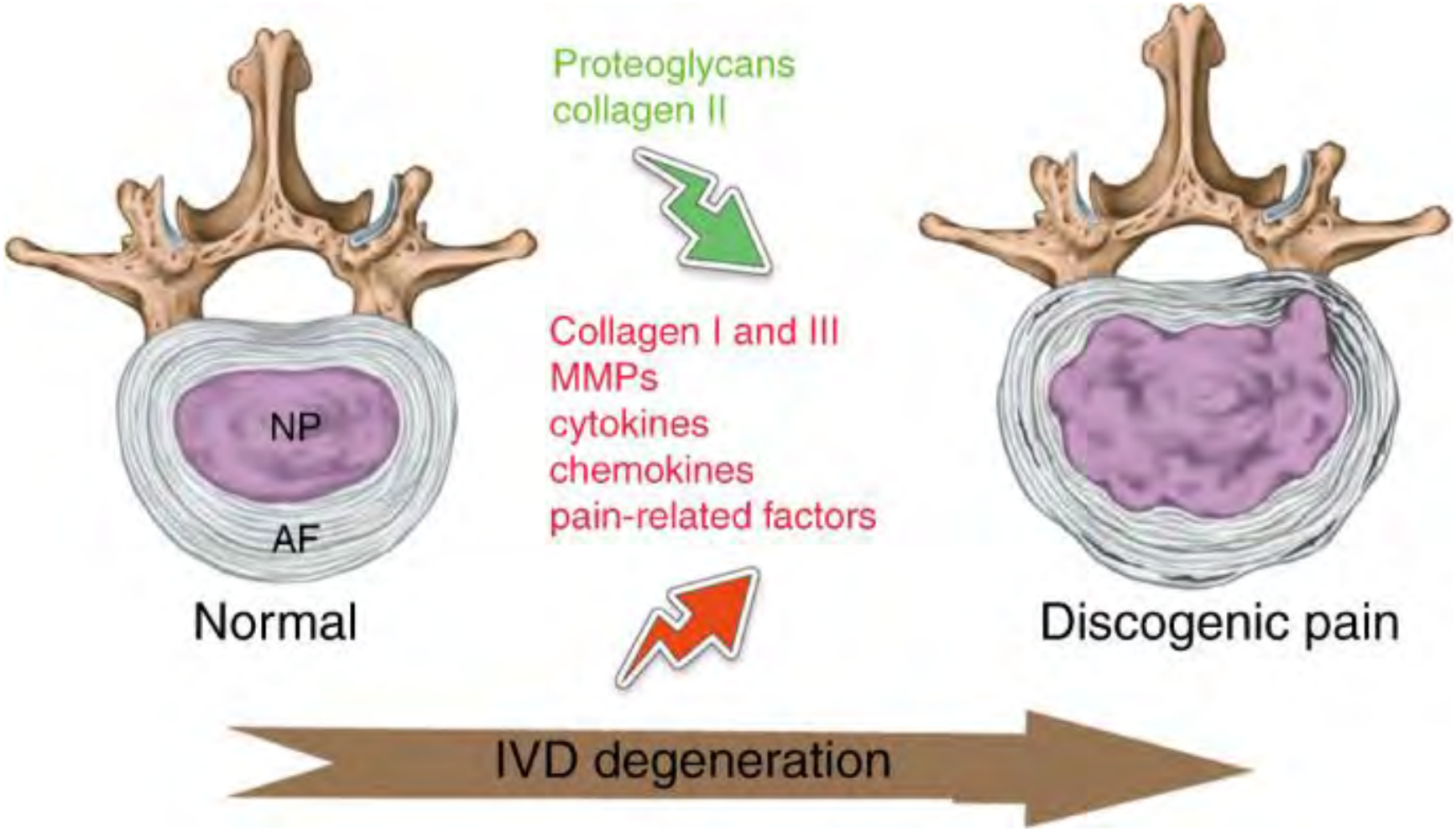
Gut-disc axis: A cause of intervertebral disc degeneration and low back pain?. *Eur Spine J* (2022)

Gut-disc axis



Gut-disc axis: A cause of intervertebral disc degeneration and low back pain?. *Eur Spine J* (2022)

Degenerative changes in painful intervertebral discs

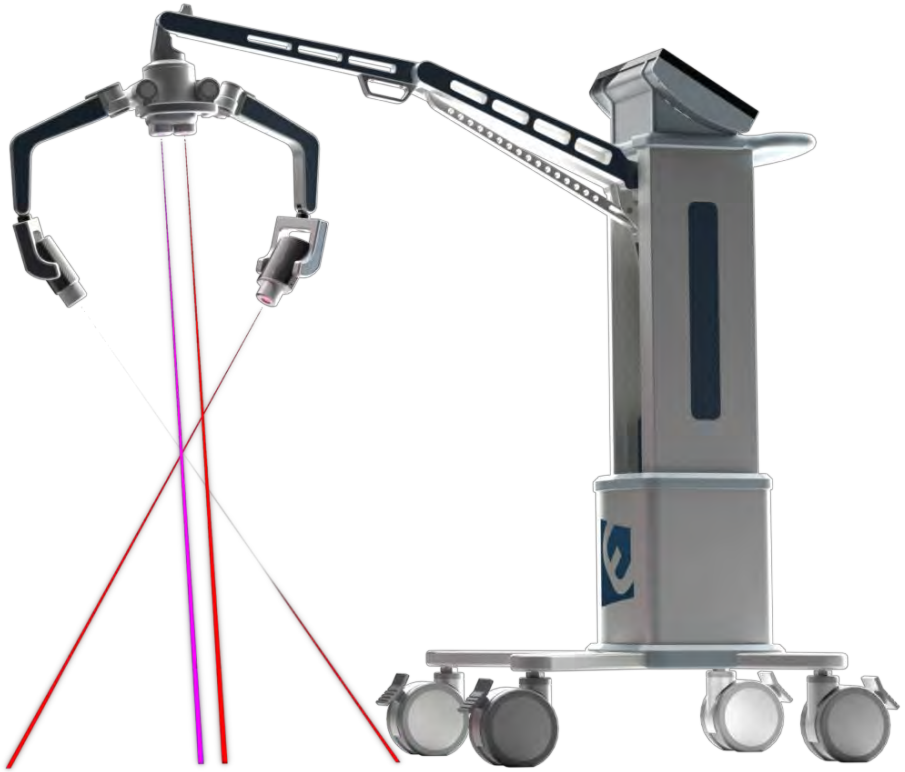


FJ Lyu, H Cui, H Pan, *et al.* **Painful intervertebral disc degeneration and inflammation: from laboratory evidence to clinical interventions**. *Bone Res*, 29 Jan 2021;9:7

Low-back FX635/405 options

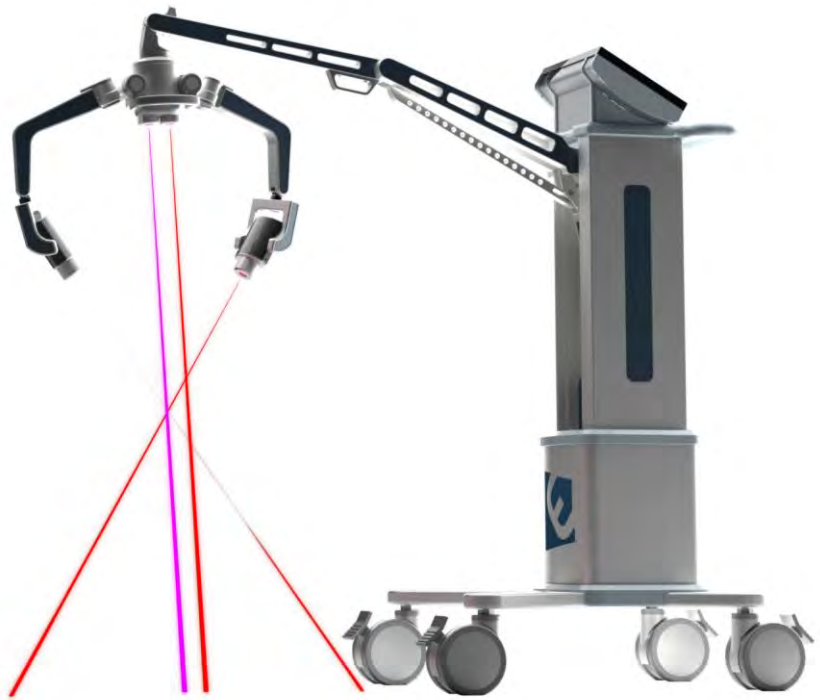
Stand-alone or synergistic with other treatments/modalities

- Stand-alone – set it and forget it
- With:
  - Myofascial release
  - E-stim
  - Exercise rehab
  - Acupuncture/dry-needling
  - Decompression
  - Taping
  - Adjustment
  - Instrument-assisted soft tissue mobilization
  - Modalities



NMDA glutamate receptors and pain

- FX405 can “scan”
- 405 violet light effectively targets NMDA glut receptor sites in the peripheral and CNS modulating sensitization of WDR’s and other effect of glutamate excitotoxicity
- Perfect for back pain



Core Lock-In

4, 9, 33, 60
30 sec. each side

The Plank

Bird Dogs

Glucosamine & Chondroitin Sulfate Combined May Support Overall Musculoskeletal Integrity

BMC Complement Altern Med. 2003 Jun 10;3:2. Epub 2003 Jun 10.

Glucosamine and chondroitin sulfate supplementation to treat symptomatic disc degeneration: biochemical rationale and case report.

van Blitterswijk WJ, van de Nes JC, Wuisman PI.
Division of Cellular Biochemistry, The Netherlands Cancer Institute, Antoni van Leeuwenhoek Hospital, Plesmanlaan 121, 1066CX Amsterdam, The Netherlands. w.v.blitterswijk@nki.nl

BACKGROUND: Glucosamine and chondroitin sulfate preparations are widely used as food supplements against osteoarthritis, but critics are skeptical about their efficacy, because of the lack of convincing clinical trials and a reasonable scientific rationale for the use of these nutraceuticals. Most trials were on osteoarthritis of the knee, while virtually no documentation exists on spinal disc degeneration. The purpose of this article is to highlight the potential of these food additives against cartilage degeneration in general, and against symptomatic spinal disc degeneration in particular, as is illustrated by a case report. The water content of the intervertebral disc is a reliable measure of
objectively determined by Magnetic Resonance Imaging (
glucosamine and chondroitin sulfate for two years associa
in a case of symptomatic spinal disc degeneration. We pro
efficacy of these nutraceuticals. They are bioavailable to c
biosynthesis and inhibit the breakdown of their extracellu
**suggests that long-term glucosamine and chondroitin su
disc degeneration, particularly at an early stage.** Howeve
trials with these food supplements, in which disc de-/rege
number of biochemical reasons (that mechanistically nee
may have cartilage structure- and symptom-modifying eff
osteoarthritis in general.

"The case suggests that long-term glucosamine and chondroitin sulfate intake may counteract symptomatic spinal disc degeneration."

BMC Complement Altern Med. 2003 Jun 10;3:2

Omega-3 fatty (n3-FA) acid supplementation reduces intervertebral disc degeneration (IVD)

- EPA/DHA in a 2:1 ratio used
- 4-6 gram needed
- Reduction of blood AA/EPA ratios from 40 to 20 was demonstrated after 1 month of daily supplementation

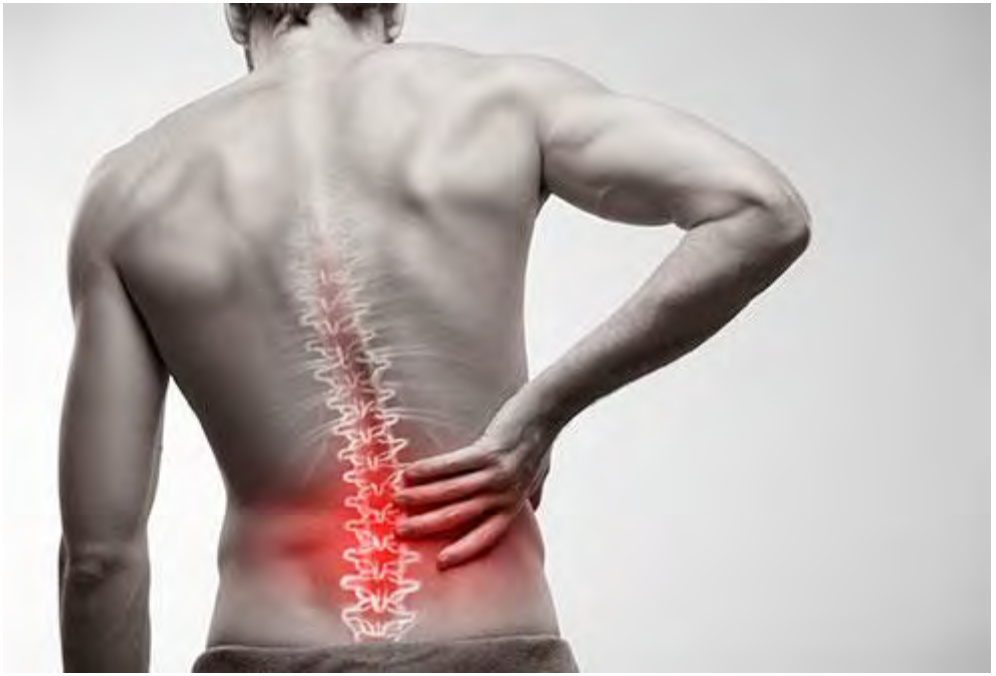
Conclusion:

- n-3 FA dietary supplementation reduces systemic inflammation by lowering AA/EPA ratios
- Has potential protective effects on the progression of spinal disc degeneration

NaPier Z, Kanim LEA, Arabi Y, et al. *Med Sci Monit,* 2019 Dec 14;25:9531-9537

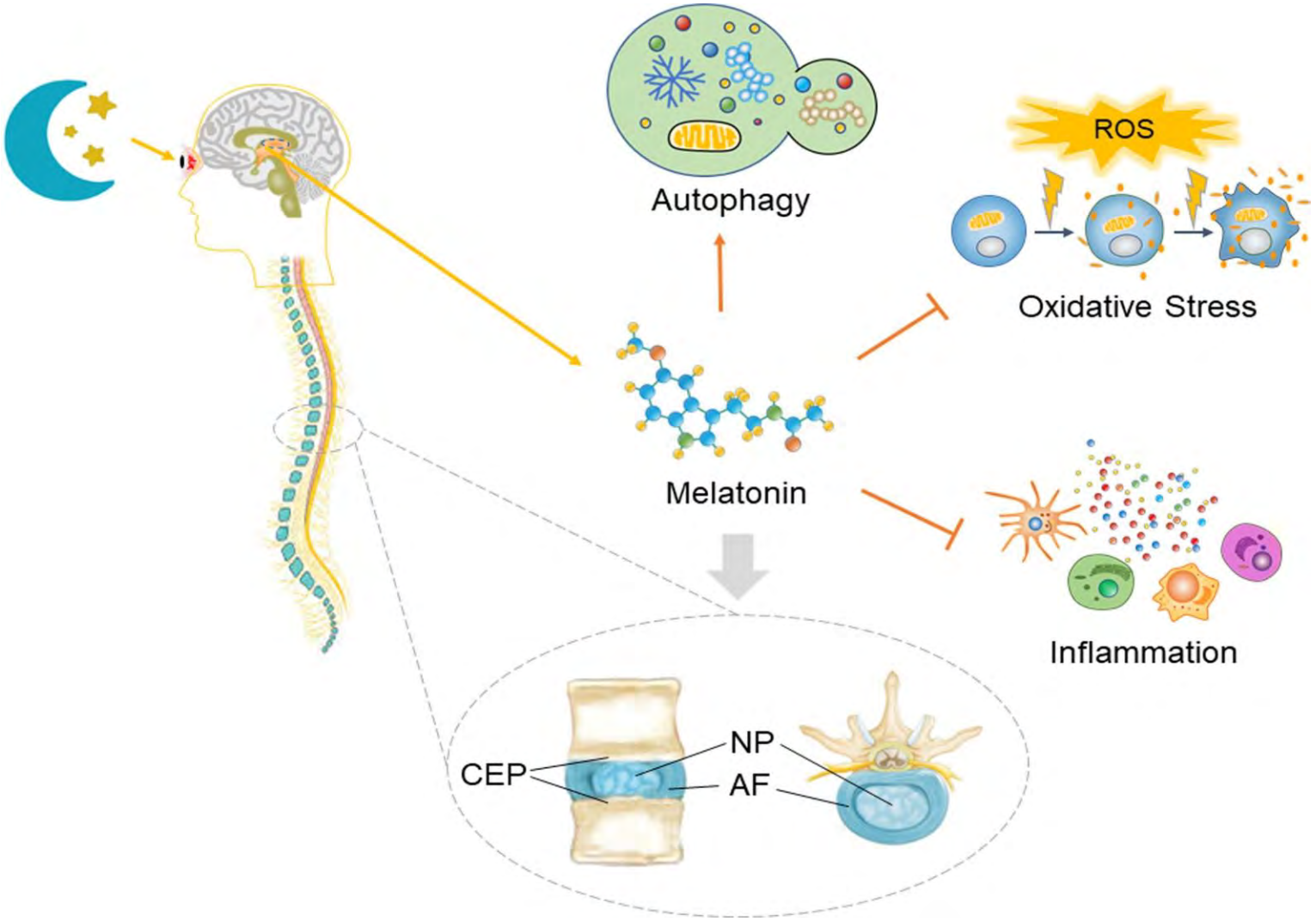
Lower back pain (LBP)

Conclusion: Subjects with vitamin D deficiency or insufficiency were 2.3 times more likely to exhibit LBP than subjects with normal vitamin D3 concentration



BMC research notes. Dec 1, 2019

The effects of melatonin on IVD cells during intervertebral disc ageing and degeneration



The potential role of melatonin in retarding intervertebral disc ageing and degeneration: A systematic review. *Ageing Research Reviews,* 2021;70:101394

Melatonin

Highlights:

- Can effectively alleviate intervertebral disc ageing and degeneration
- Inhibits disc cell apoptosis and degeneration in multiple ways
- Promotes matrix anabolism in intervertebral disc cells
- Resists oxidative stress, regulates autophagy, and inhibits inflammation

Z Cheng, Q Xiang, J Wang, Y Zhang. **The potential role of melatonin in retarding intervertebral disc ageing and degeneration: A systematic review**. *Ageing Research Reviews,* 2021;70:101394

L. Paracasei S16 and lumbar disc herniation

Results:

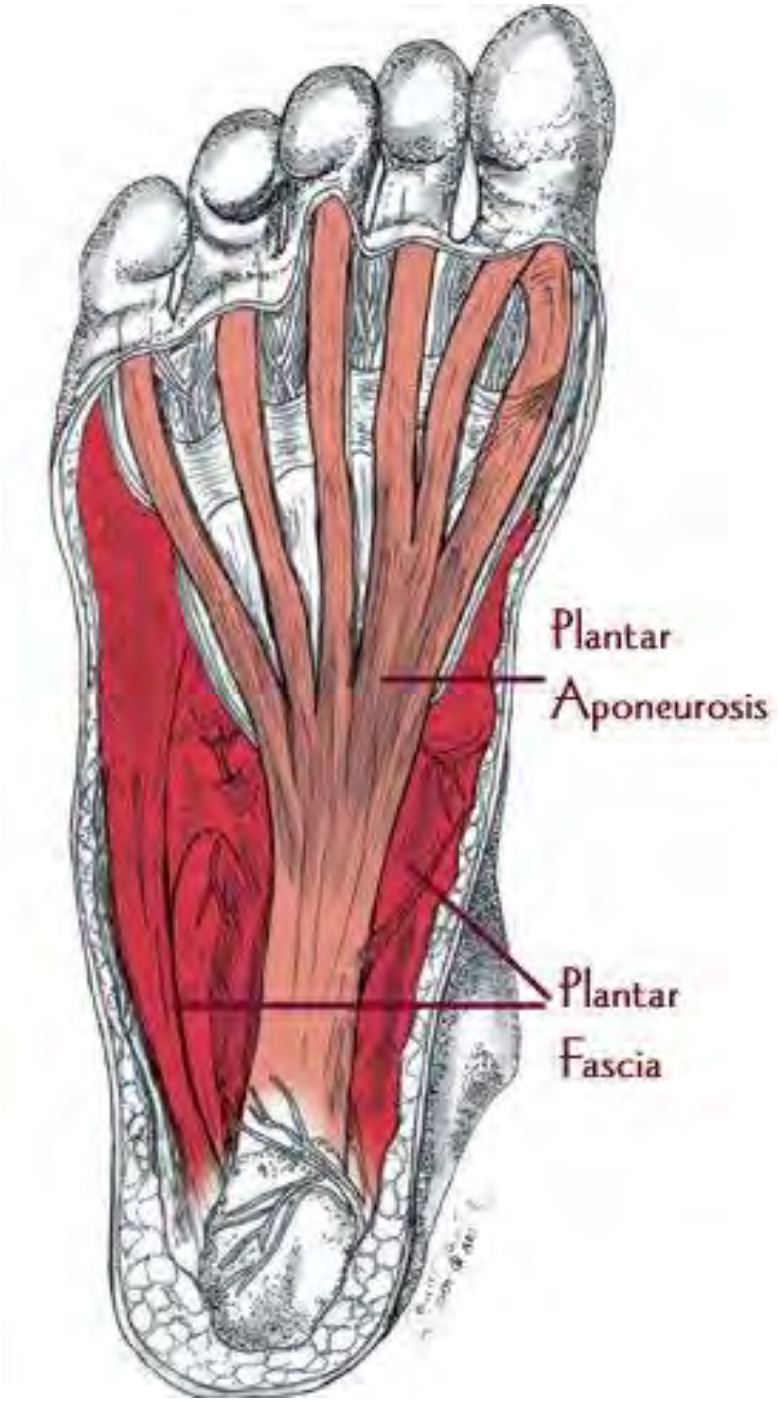
- *L. paracasei S16* treatment improved behavior
- Increased cell proliferation
- Decreased apoptosis in LDH mice
- Alleviated aberrant inflammation response
- Decreased serum metabolites involved in linoleic acid metabolism, alanine, aspartate, and glutamate

L. paracasei S16 can improve inflammation response, alter gut microbiota

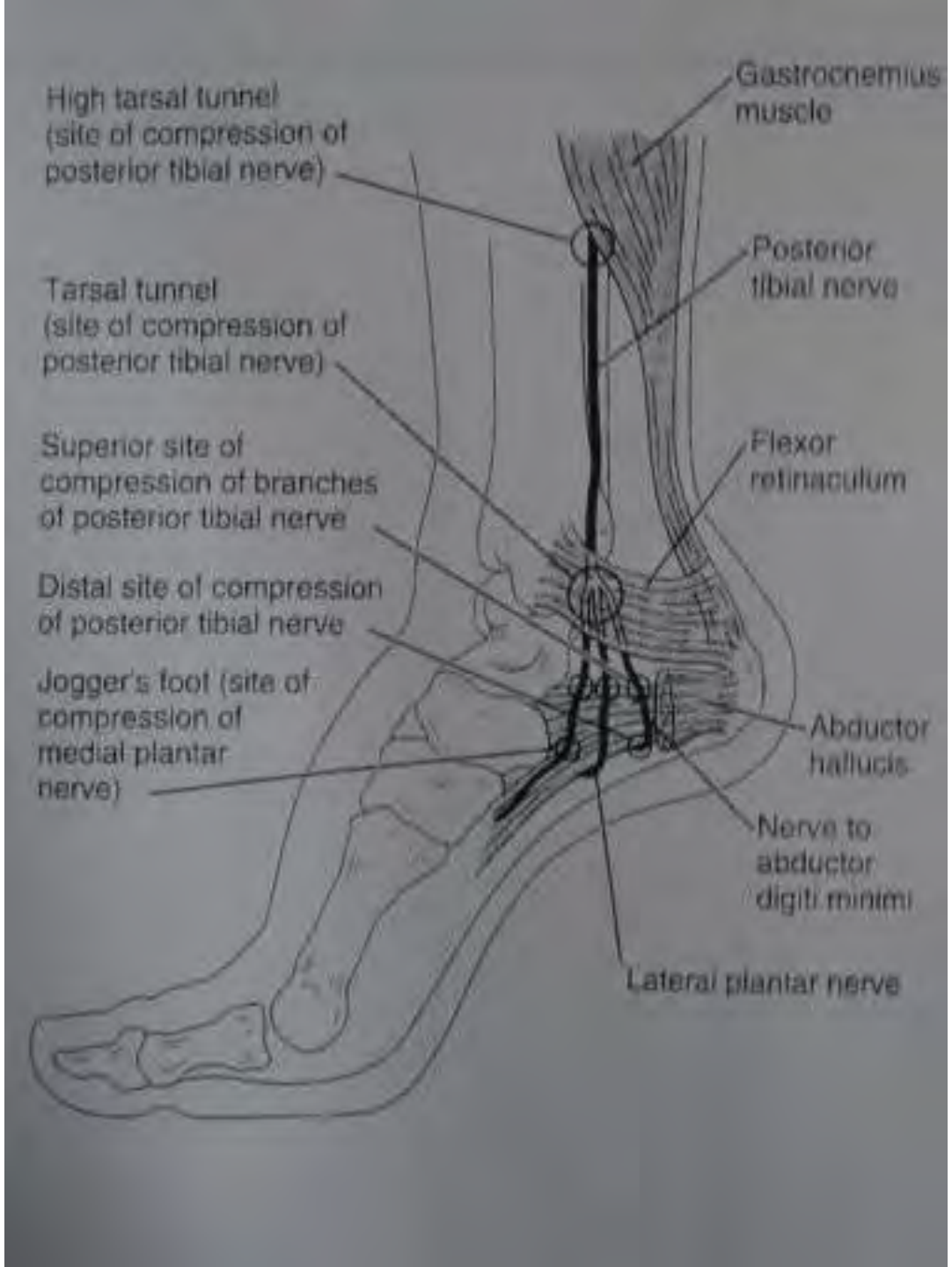
Wang Z, Wu H, Chen Y, et al. ***Lactobacillus paracasei S16* Alleviates Lumbar Disc Herniation by Modulating Inflammation Response and Gut Microbiota**. *Front Nutr*. 2021;8:701644. Published 2021 Aug 10

Plantar Fasciitis

- Facts
- Symptoms
- What it is
- What causes it
- Clinical assessment
- Treatment



Tarsal Tunnel Syndrome



Entrapment Neuropathies of the Foot and Ankle

Key points:

- Posterior tarsal tunnel syndrome – result of compression of posterior tibial nerve
- Anterior tarsal tunnel syndrome (entrapment of deep peroneal nerve) typically presents with pain radiating to first dorsal web space
- Distal tarsal tunnel syndrome results from entrapment of first branch of lateral plantar nerve – often misdiagnosed initially as plantar fasciitis

E. Ferkel, et al. *Clin Sports Med*. Oct 2015;p.791-801

Baxter Neuropathy

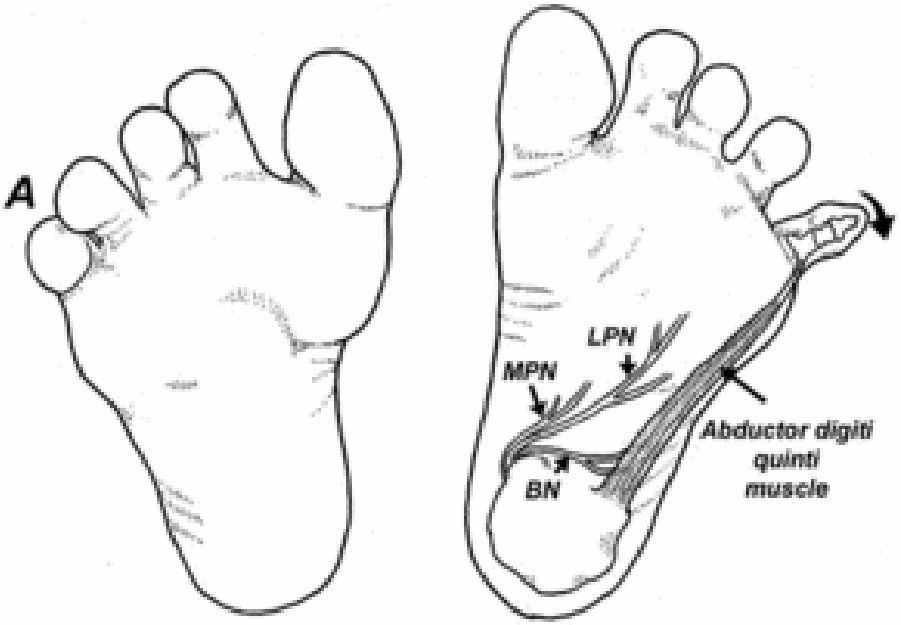
- Is a nerve entrapment syndrome resulting from the compression of the inferior calcaneal nerve (ICN)
- ICN is the first branch of the lateral plantar nerve which courses in a medial to lateral direction (between Abductor hallucis and medial calcaneal tuberosity)
- Approximately 20% of cases of pain in the medial region of the heel are associated with neuropathy of that nerve

Radiologia Brasileira 2015;48(6):368-372

Baxter's Neuropathy (cont'd)
Plantar fascia vs. Baxter

- **Test 1: Spread your toes** (motor division of nerve supplies – abductor digiti minimi)
  - Look to see if small toe can move (can't abduct little toe)

- **Test 2: Dorsiflexion and abduction** (causes ischemic to lateral plantar nerve)
  - Hold for 30 seconds to see reproduction of symptoms



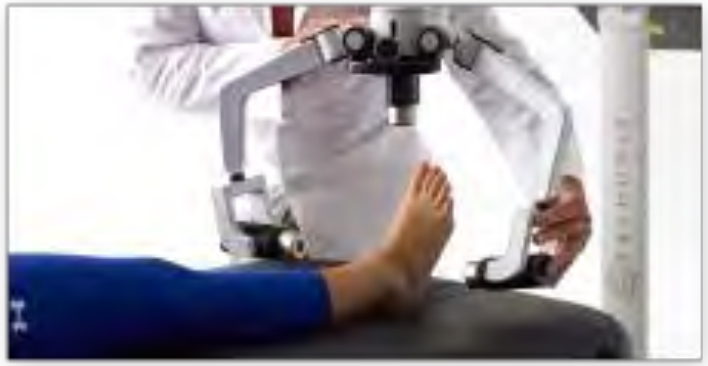
The prevalence of tarsal tunnel syndrome in patients with lumbosacral radiculopathy

- **Conclusion:** Findings suggest prevalence of TTS significant in patients with LR. More caution should be paid when diagnosing and managing patients with LR due to possible existence of TTS, as their management strategies are quite different

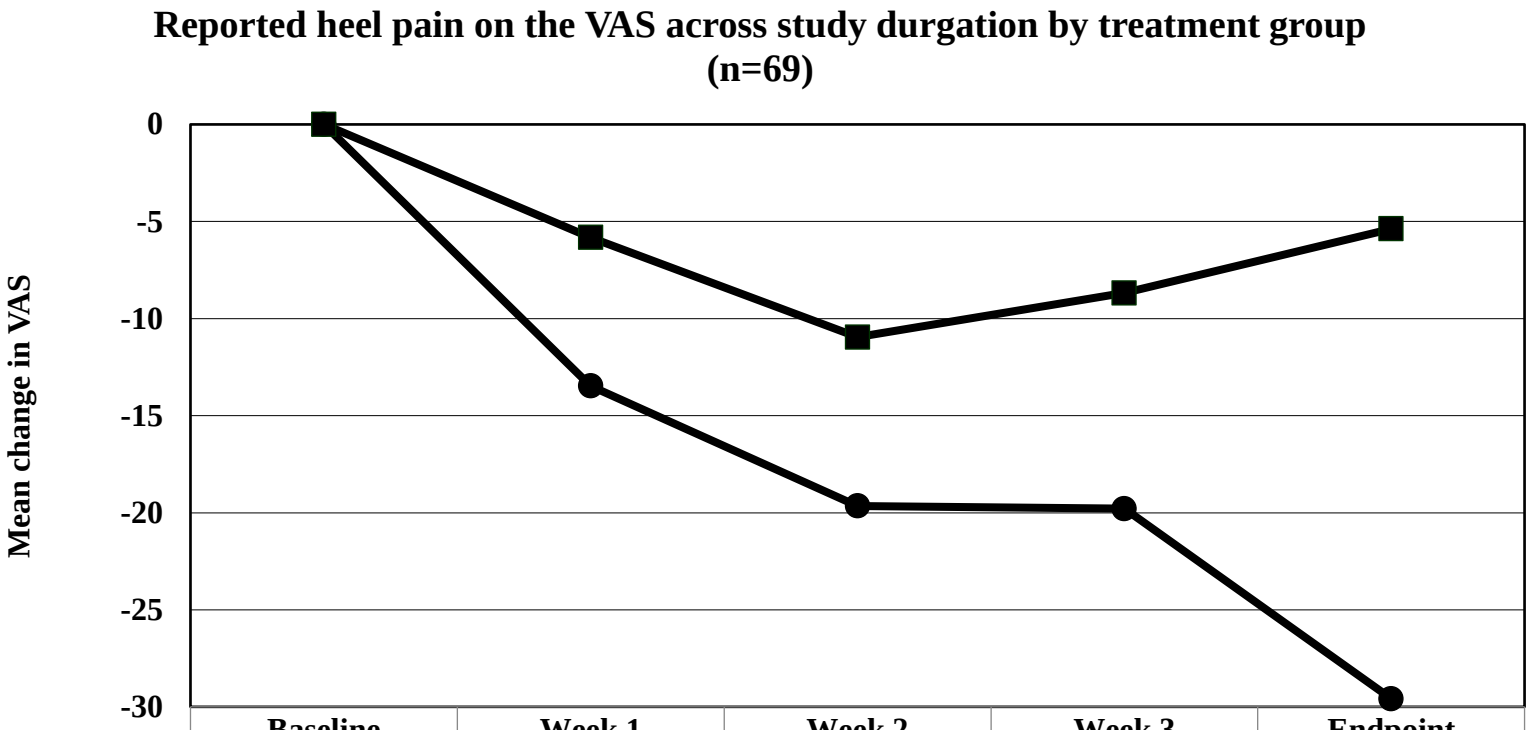
Zheng C. et al. *Eur Spine J*. 2015

Plantar Fasciitis Laser Protocol

- 2 treatments a week for 3 weeks
- Area- top of foot (Dorsal Aspect), the myofascial junction of the heel and the plantar aspect of the heel
- All treated simultaneously. 10 minutes per area



Chronic Heel Pain
Plantar Fasciitis



	Baseline	Week 1	Week 2	Week 3	Endpoint
Test	0	-13.47	-19.65	-19.79	-29.58
Placebo	0	-5.82	-10.96	-8.68	-5.38

Chronic Heel Pain
Plantar Fasciitis

Low-Level Laser Therapy for the Treatment of Chronic Plantar Fasciitis
A Prospective Study

James R. Jastifer, MD[1],Fernanda Catena, MD[2],Jesse F. Doty, MD[3],Faustin Stevens, MD[4],Michael J. Coughlin, MD[1]

Abstract

Background: Plantar fasciitis affects nearly 1 million people annually in the United States. Traditional nonoperative management is successful in about 90% of patients, usually within 10 months. Chronic plantar fasciitis develops in about 10% of patients and is a difficult clinical problem to treat. A newly emerging technology, low-level laser therapy (LLLT), has demonstrated promising results for the treatment of acute and chronic pain.

Methods: Thirty patients were administered LLLT and completed 12 months of follow-up. Patients were treated twice a week for 3 weeks for a total of 6 treatments and were evaluated at baseline, 2 weeks post procedure, and 6 and 12 months post procedure. Patients completed the Visual Analog Scale (VAS) and Foot Function Index (FFI) at study follow-up periods.

Results: Patients demonstrated a mean improvement in heel pain VAS from 67.8 out of 100 at baseline to 6.9 out of 100 at the 12-month follow-up period. Total FFI score improved from a mean of 106.2 at baseline to 32.3 at 12 months post procedure.

Conclusion: Although further studies are warranted, this study shows that LLLT is a promising treatment of chronic plantar fasciitis.

Level of Evidence: Level 4, case series.

plantar fasciitis heel pain laser therapy

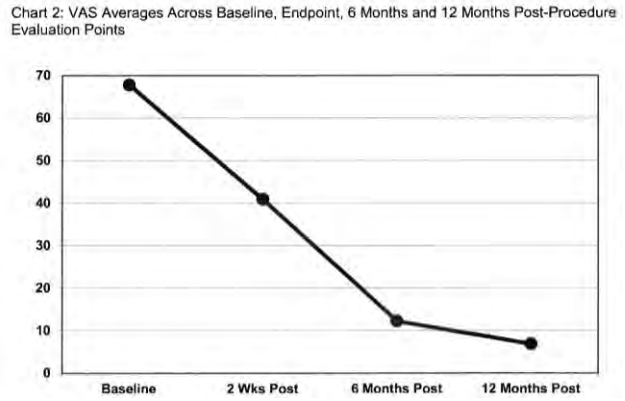
Plantar heel pain is one of the most common pathologies of the foot, accounting for up to 15% of foot-related symptoms presenting to physicians and 1% of all visits to orthopedic surgeons.[30] The clinical manifestations can be disabling, and despite its high incidence, the specific cause of plantar fasciitis is poorly understood; it is likely multifactorial and may be associated with systemic disease, local changes to the plantar fascia tissue, or altered foot and ankle biomechanics.[6,14,20,24,32]

The choice of nonoperative treatment is largely up to the physician. Numerous treatment options exist, including stretching, night splints, orthotics, casting, steroid injections, and anti-inflammatory medications. There is limited high-level evidence to support one treatment over another.[11] The treatment of plantar fasciitis can be frustrating, yet about 90% of patients will respond favorably to nonoperative treatment, usually within 10 months.[10,12]

Failure of these measures occurs in about 10% of patients, resulting in chronic plantar fasciitis and a difficult clinical problem. There is also limited high-level evidence guiding the treatment of this group of patients. A recent study showed that up to 55% of foot and ankle surgeons would consider surgery for the treatment of plantar fasciitis refractory to 10 months of nonoperative management.[11]

Short of surgery, a new treatment for chronic plantar fasciitis is low-level laser therapy (LLLT), which has been used extensively in other areas of the body. It has become increasingly popular because it is painless, is noninvasive, and has shown short-term efficacy in the treatment of plantar fasciitis.[18,21] The purpose of this clinical study was to determine the effectiveness of LLLT in the treatment of chronic plantar fasciitis.

months. Thirty of the 34 patients elected to enroll and completed 12 months of follow-up. The original 16 treatment group participants had already received treatment and so were followed to the 12-month endpoint. The 14 from the original placebo group were converted to a treatment group, administered treatment, and followed to the 12-month endpoint.



Publication

- ASLMS abstract

EVALUATION OF LOW-LEVEL LASER THERAPY AT 635 nm FOR THE TREATMENT OF CHRONIC PLANTAR FASCIITIS: A PLACEBO-CONTROLLED, RANDOMIZED STUDY

Mike Coughlin, Faustin Stevens, Jesse Doty, Kerry Zang, Ryan Maloney

Alphonsus Coughlin Foot and Ankle Clinic, Boise, ID; Arizona Institute of Footcare Physicians, Mesa, AZ; Phoenix, AZ

Background: Plantar fasciitis affects close to one million people in the United States. A majority of cases are successfully treated with conservative therapies; however, roughly 10% of cases require surgical intervention. A newly emerging technology, low-level laser therapy (LLLT), has demonstrated promising results for the treatment of acute and chronic plantar fasciitis. LLLT modulates cell function, yielding analgesic and regenerative effects.

Study: Sixty-nine subjects qualified and were enrolled, from 09/2011 to 06/2013, in a placebo-controlled, randomized, double-blind, multi-center study evaluating LLLT for the treatment of unilateral chronic fasciitis. Volunteer participants were treated twice weekly for three weeks, for a total of six treatments and were evaluated at five separate time points: pre-procedure; procedure weeks 1, 2, and 3; and, on post-procedure days 21 and 35. Degree of pain was recorded using a visual analog scale (VAS), with zero representing "no pain" and 100 representing the "worst pain imaginable". Doppler ultrasound was performed on the

Results: Plantar fascia thickness was significantly reduced in test group subjects, but not in sham participants

"Satisfied" responses between LLLT and sham-treated patients ($P < 0.0001$).

Conclusion: Although further studies are warranted, these data demonstrate that LLLT is a promising treatment for plantar fasciitis. No adverse events were reported.

Low-Level Laser Therapy

- Achilles tendinosis patients who underwent eccentric exercises together with low level laser therapy showed decreased pain intensity, morning stiffness, tenderness to palpation, active dorsiflexion, and crepitus with no side effects as compared to those who underwent eccentric exercises only

Stergioulas A, Stergioula M, et al. Effects of low-level laser therapy and eccentric exercises in the treatment of recreational athletes with chronic Achilles tendinopathy. *Am J Sports Med*. 2008;36 95 0:881-887

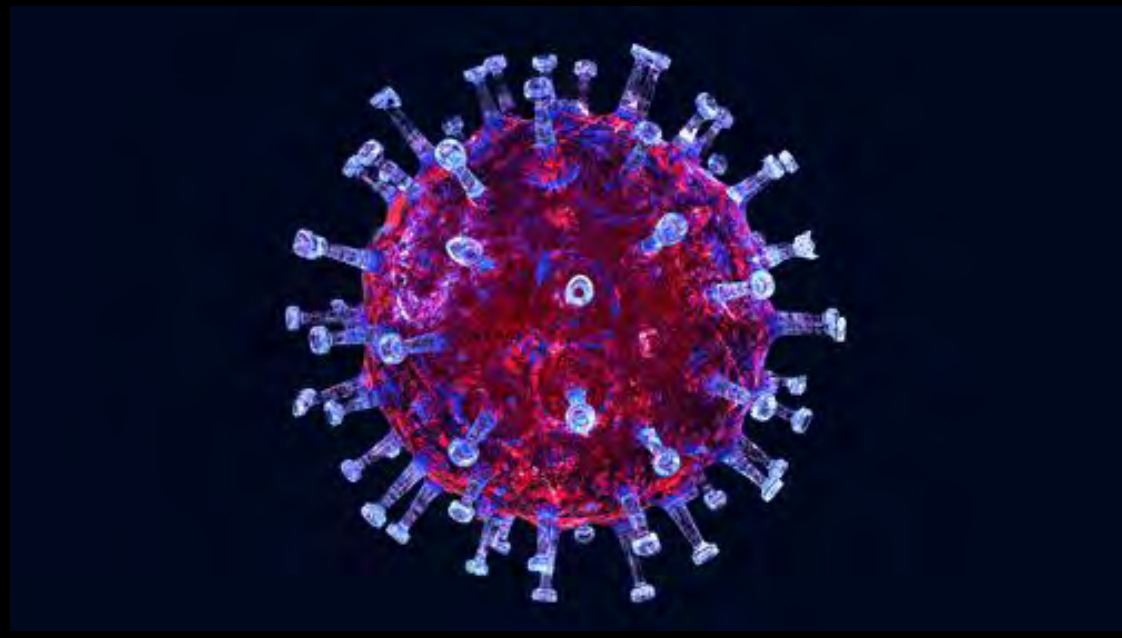
Efficacy of LLLT with lower extremity tendinopathy or PF

Conclusion:

LLLT significantly reduces pain and disability in lower extremity tendinopathy and plantar fasciitis in the short and medium term

Naterstad IF, Joensen J, Bjordal JM, et al. **Efficacy of low-level laser therapy in patients with lower extremity tendinopathy or plantar fasciitis: systematic review and meta-analysis of randomised controlled trials**. *BMJ Open*. 2022 Sep 28;12(9):e059479

You can't control the virus, but you can *control* the *host*



“You are only as young as your immune system”

Accelerated biological aging in COVID-19 patients

- Accelerated epigenetic aging associated with risk of SARS-CoV-2 infection and developing severe COVID-19
- Accumulation of epigenetic aging from COVID-19 may contribute to post COVID-19 syndrome among survivors

X Cao, W Li, T Wang, T. *et al.* **Accelerated biological aging in COVID-19 patients.** *Nat Commun,* 19 April 2022;13(2135)

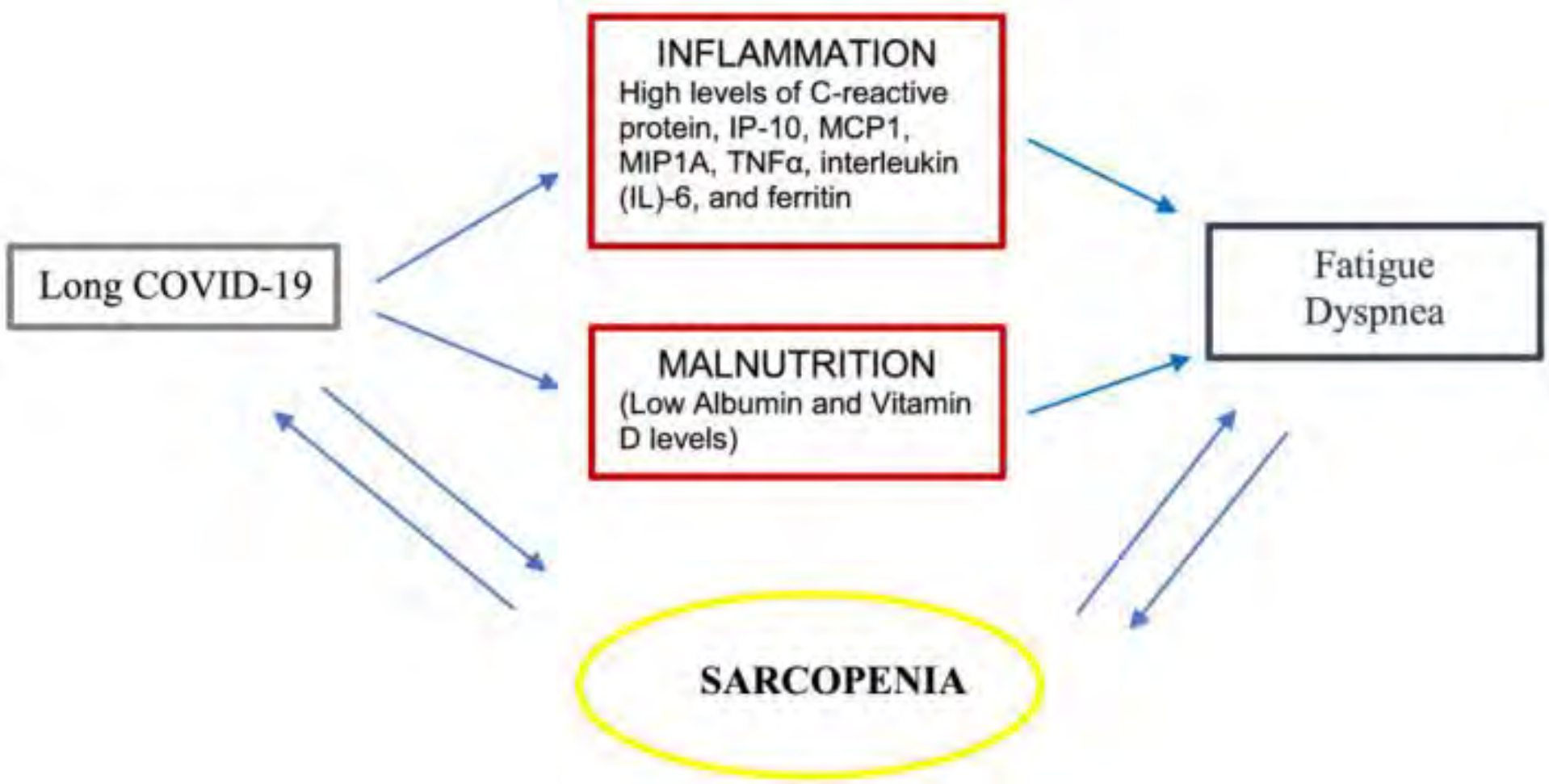
Telomere shortening/COVID-19

Results:

- Show a consistent biological age increase in post-COVID-19 population
- Significant telomere shortening parallels this finding in post-COVID-19 cohort compared with COVID-19-free subjects
- ACE2 expression was decreased in post-COVID-19 patients compared with COVID-19-free population

Mongelli A, Barbi V, Gottardi Zamperla M, et al. **Evidence for Biological Age Acceleration and Telomere Shortening in COVID-19 Survivors**. *Int J Mol Sci*. 2021;22(11):6151

Interaction between COVID-19 and sarcopenia



Sarcopenia as potential biological substrate of long COVID-19 syndrome: prevalence, clinical features, and risk factors. *J Cachexia Sarcopenia Muscle*, 14 June 2022;13(4):1974-82

Short-term impact of COVID-19 pandemic on low back pain

Conclusion:

Although prevalence of LBP did not change at the first months of COVID-19 pandemic, LBP-induced impairment in daily activities and pain intensity was higher when compared to before the pandemic

Caputo EL, Ferreira PH, Feter N, et al. **Short-term impact of COVID-19 pandemic on low back pain: data from the PAMPA Cohort, Brazil**. *BMC Public Health*. 2023;23(1):44. Published 2023 Jan 6

COVID ups diabetes risk 40% a year later

Risks and burdens of incident diabetes in long COVID: a cohort study

Yan Xie, Ziyad Al-Aly

Summary

Background There is growing evidence suggesting that beyond the acute phase of SARS-CoV-2 infection, people with COVID-19 could experience a wide range of post-acute sequelae, including diabetes. However, the risks and burdens of diabetes in the post-acute phase of the disease have not yet been comprehensively characterised. To address this knowledge gap, we aimed to examine the post-acute risk and burden of incident diabetes in people who survived the first 30 days of SARS-CoV-2 infection.

Methods In this cohort study, we used the national databases of the US Department of Veterans Affairs to build a cohort of 181 280 participants who had a positive COVID-19 test between March 1, 2020, and Sept 30, 2021, and survived the first 30 days of COVID-19; a contemporary control (n=4 118 441) that enrolled participants between March 1, 2020, and Sept 30, 2021; and a historical control (n=4 286 911) that enrolled participants between March 1, 2018, and Sept 30, 2019. Both control groups had no evidence of SARS-CoV-2 infection. Participants in all three comparison groups were free of diabetes before cohort entry and were followed up for a median of 352 days (IQR 245–406). We used inverse probability weighted survival analyses, including predefined and algorithmically selected high dimensional variables, to estimate post-acute COVID-19 risks of incident diabetes, antihyperglycaemic use, and a composite of the two outcomes. We reported two measures of risk: hazard ratio (HR) and burden per 1000 people at 12 months.

Findings In the post-acute phase of the disease, compared with the contemporary control group, people with COVID-19 exhibited an increased risk (HR 1·40, 95% CI 1·36–1·44) and excess burden (13·46, 95% CI 12·11–14·84, per 1000 people at 12 months) of incident diabetes; and an increased risk (1·85, 1·78–1·92) and excess burden (12·35, 11·36–13·38) of incident antihyperglycaemic use. Additionally, analyses to estimate the risk of a composite endpoint of incident diabetes or antihyperglycaemic use yielded a HR of 1·46 (95% CI 1·43–1·50) and an excess burden of 18·03 (95% CI 16·59–19·51) per 1000 people at 12 months. Risks and burdens of post-acute outcomes increased in a graded fashion according to the severity of the acute phase of COVID-19 (whether patients were non-hospitalised, hospitalised, or admitted to intensive care). All the results were consistent in analyses using the historical control as the reference category.

Interpretation In the post-acute phase, we report increased risks and 12-month burdens of incident diabetes and antihyperglycaemic use in people with COVID-19 compared with a contemporary control group of people who were enrolled during the same period and had not contracted SARS-CoV-2, and a historical control group from a pre-pandemic era. Post-acute COVID-19 care should involve identification and management of diabetes.

Yan Xie, Ziyad Al-Aly. **Risks and burdens of incident diabetes in long COVID: a cohort study.** *The Lancet Diabetes & Endocrinology*, 21 March 2022

Influence of nutritional intakes in Japan and the US on COVID-19 infection

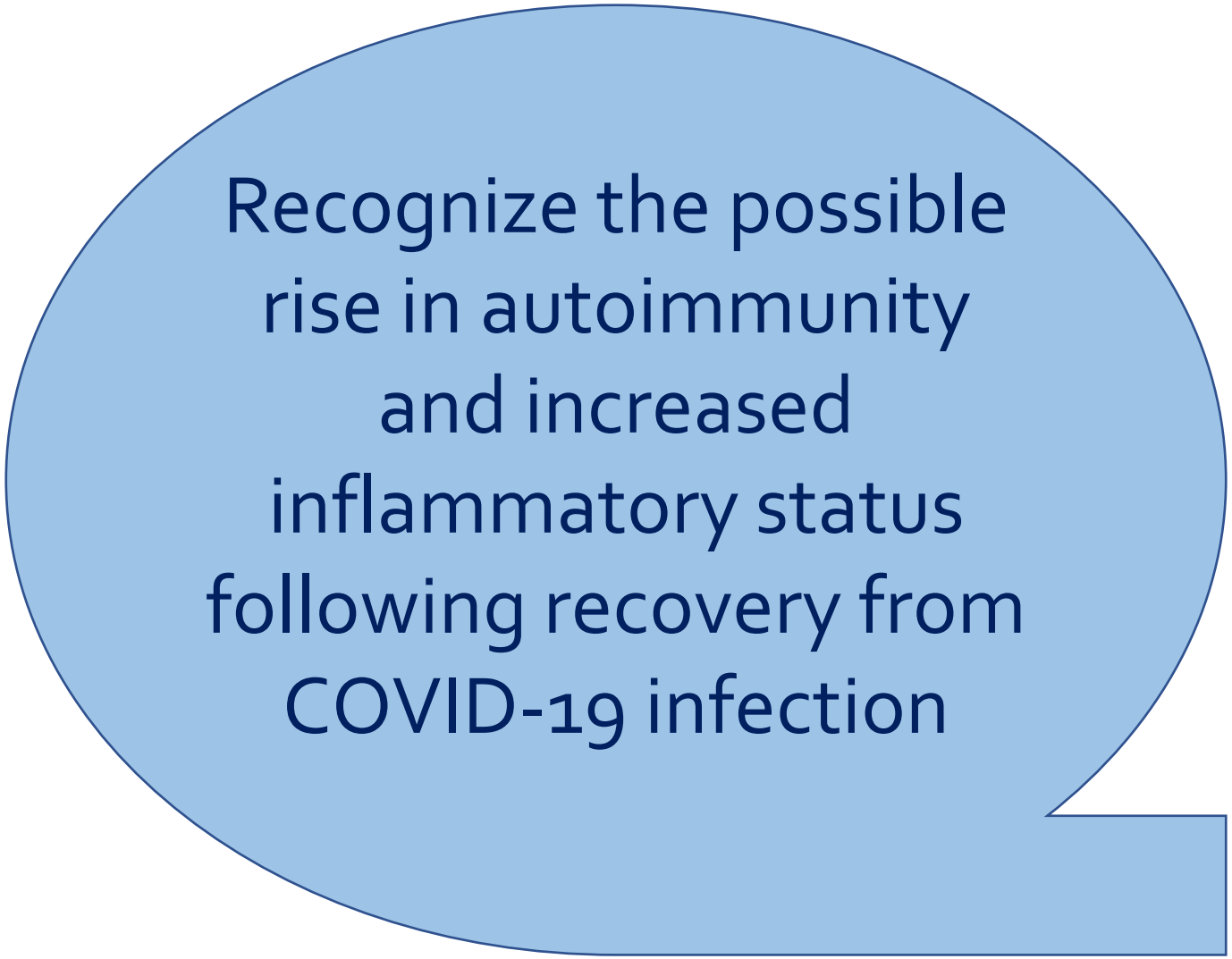
- COVID cases - 12.1 X higher in the US than Japan
- Death - 17.4 X higher in the US than Japan

Diet

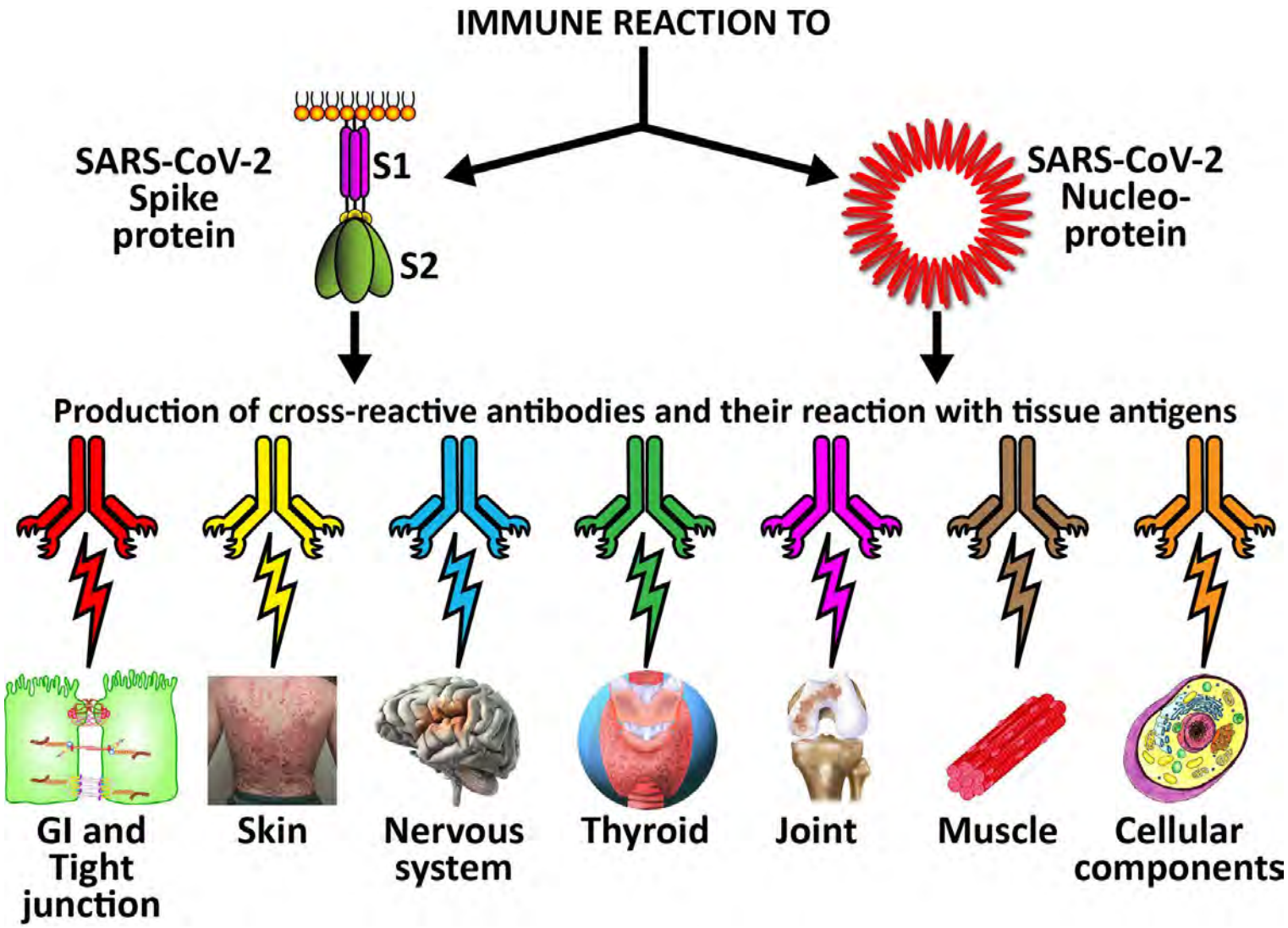
- 1.5 X more saturated fat, less EPA/DHA consumed in the US than in Japan
- US consumes more: beef 396%, sugar and sweeteners 235%
- Japan more: fish 44.3%, rice 11.5%, tea 54.7%
- Prevalence of obesity:
  - American men – 7.4 X greater
  - American women – 10 X greater

Kagawa Y. **Influence of Nutritional Intakes in Japan and the United States on COVID-19 Infection**. *Nutrients.* 2022 Feb 1;14(3):633

Let's Talk

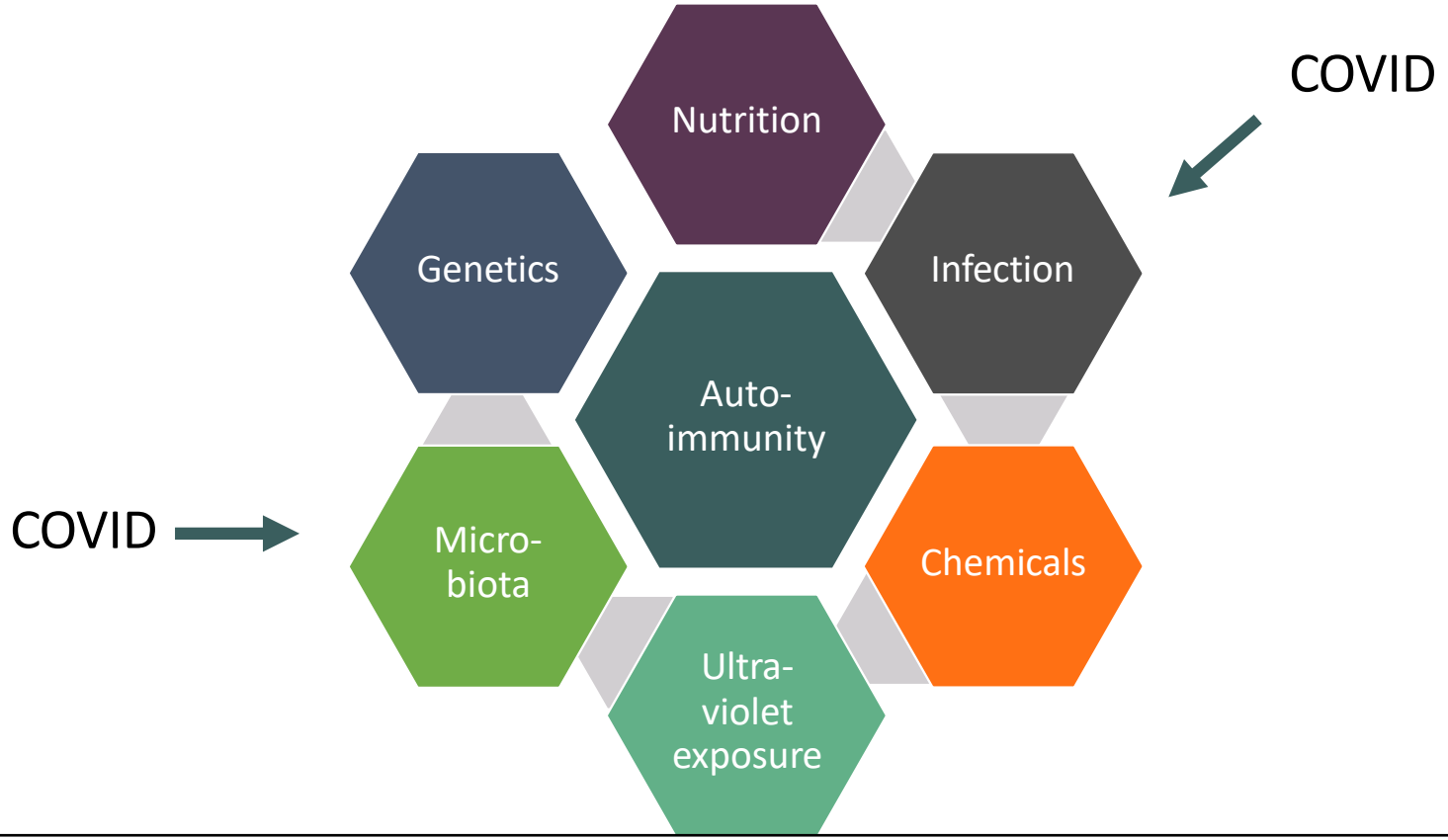

Recognize the possible rise in autoimmunity and increased inflammatory status following recovery from COVID-19 infection

Possible relationship between SARS-CoV-2 proteins and autoimmune target proteins



Vojdani A, Vojdani E, Kharrazian D. *Front Immunol*. 19 Jan 2021;11:3679

What is the trigger for autoimmunity?

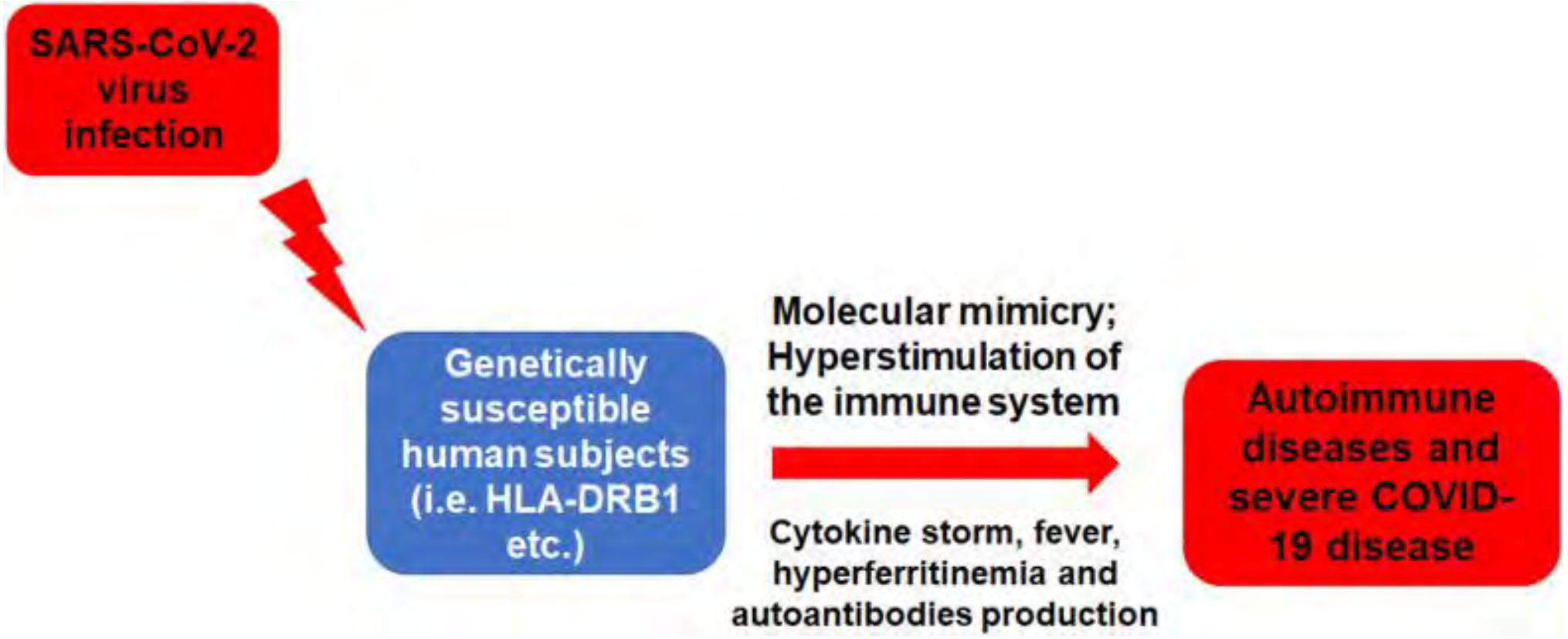


How does COVID-19 cause autoimmunity?

- Associated with overactivation of mature natural killer cells and $CD8^+$ T cells
- Infection characterized by:
  - Dysregulated B and T cells
  - Inflammatory cytokines:
    - TNF-alpha
    - IL-1
    - IL-6
  - Leukocyte and neutrophil elevated

COVID and autoimmune diseases: one and the same? *Welltheory.com,* 16 March 2023

The development of autoimmune diseases following SARS-CoV-2 infection



Halpert G, Shoenfeld Y. **SARS-CoV-2, the autoimmune virus.** *Autoimmun Rev*. 2020;19(12):102695. doi:10.1016/j.autrev.2020.102695

The immune system provides 3 levels of defense against disease-causing organisms

1

BARRIERS

Prevent entry

- Skin & mucus membranes
- Stomach acid & digestive enzymes
- Beneficial bacteria that live in the colon (the gut microbiota)

2

INNATE IMMUNITY

General defense

- WBCs, called neutrophils & macrophages, engulf & destroy foreign invaders & damaged cells

3

ACQUIRED IMMUNITY

Specific defense

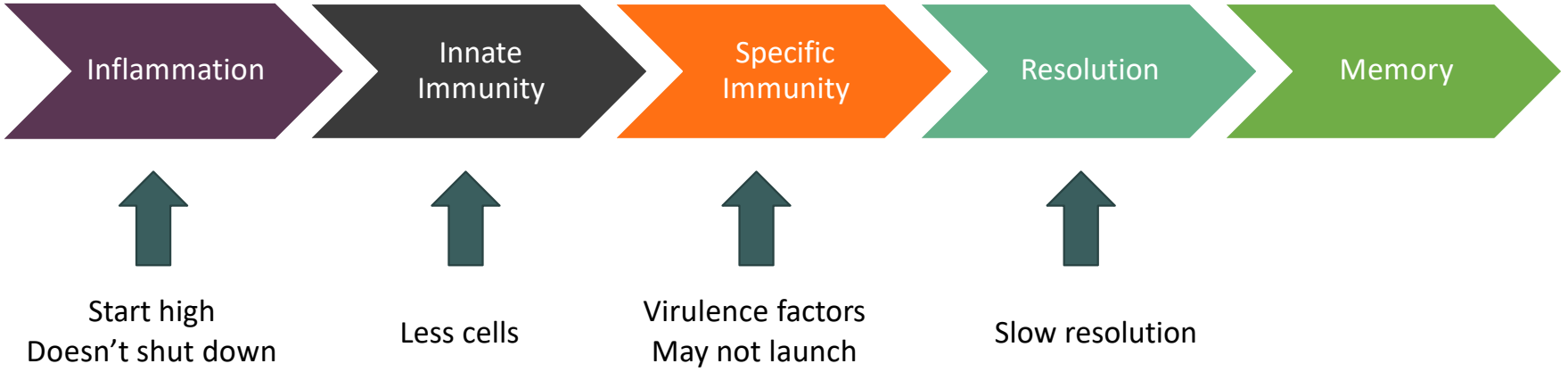
- WBCs called T lymphocytes (T cells) target & destroy infected or cancerous cells
- WBC's called B lymphocytes (B cells) & plasma cells produce antibodies that target & destroy infected or cancerous cells

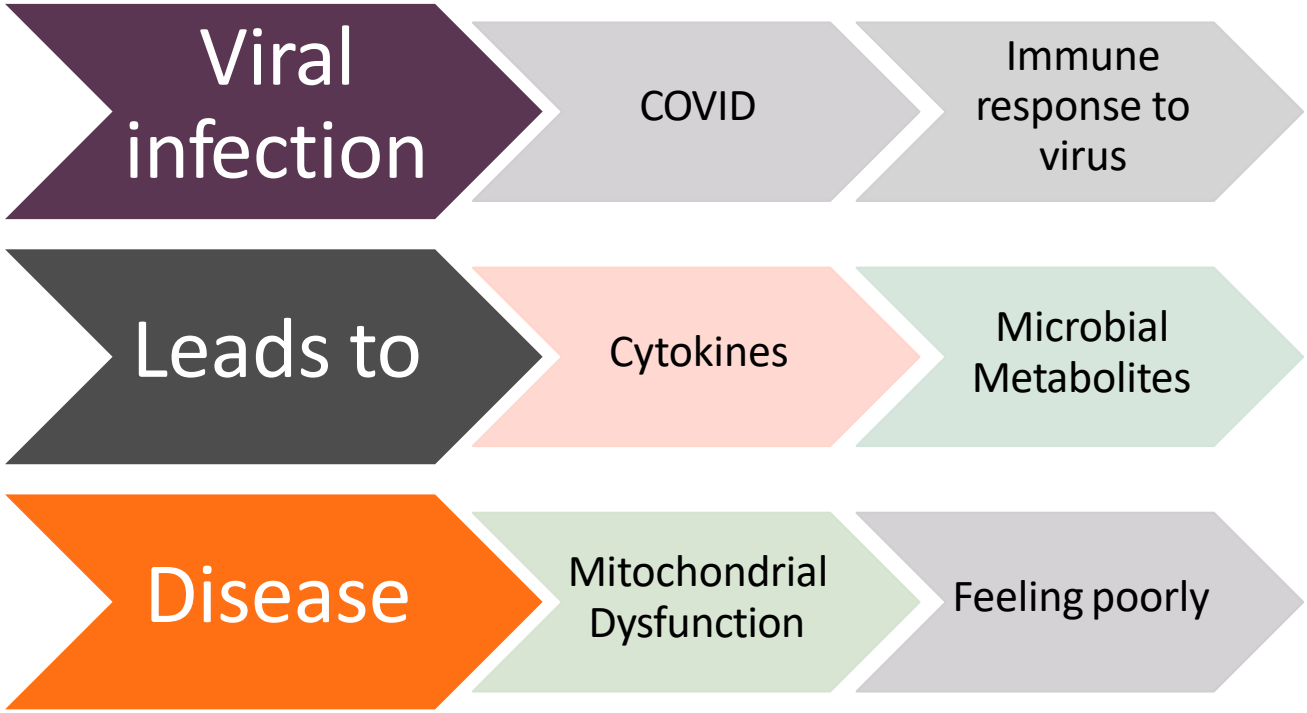
Innate vs. acquired immunity

Normal Immune Response to Infection

Immune Response to COVID

SARS-CoV2




Viral infection
COVID
Immune response to virus
Leads to
Cytokines
Microbial Metabolites
Disease
Mitochondrial Dysfunction
Feeling poorly

Immune imprinting (original antigenic sin)

A phenomenon in which the body preferentially repeats its immune response to the first variant it encountered, despite being alerted to a new variant

Immune Imprinting and Protection against Repeat Reinfection with SARS-CoV-2. *N Engl J Med*, 3 Nov 2022; 387:1716-1718

Immune imprinting/long-COVID

- MIT and Harvard
- 112 patients – neurological long-COVID symptoms:
  - Inflammation of brain with cognitive deficits
- Found:
  - Underwhelming antibodies to COVID
  - Overwhelming antibody response to other coronaviruses

Findings suggest – immune imprinting can cause neurological long-COVID

M Spatola, N Nziza, WY Jung, et al. **Neurologic sequalae of COVID-19 are determined by immunologic imprinting from previous Coronaviruses**. *medRxiv* 2022.11.07.22282030

IL-1β, IL-6, and TNF/long-COVID

Highlights

- PASC persists in 60% of participants up to 24 months after mild COVID-19
- PASC associated with high IL-1β, IL-6, and TNF levels but not autoantibodies
- Overactivated monocytes/macrophages are likely source of cytokine production

Schultheiß C, Willscher E, Paschold L, et al. **The IL-1β, IL-6, and TNF cytokine triad is associated with post-acute sequelae of COVID-19.** *Cell Rep Med*. 2022 Jun 21;3(6):100663

Long COVID

Definition:

The continuation or development of new symptoms 3 months after the initial SARS-CoV-2 infection, with these symptoms lasting for at least 2 months with no other explanation

WHO Europe. 7 Dec 2022

WOW!!

Estimates have long-COVID costing the US economy $3.7 trillion and growing

CNBC

Covid-19

UCLA researchers –

30% of people treated for COVID-19 developed PASC

SM Yoo, TC Liu, Y Motwani, Y. *et al.* **Factors Associated with Post-Acute Sequelae of SARS-CoV-2 (PASC) After Diagnosis of Symptomatic COVID-19 in the Inpatient and Outpatient Setting in a Diverse Cohort**. *J Gen Intern Med*, 7 Apr 2022

Cleveland clinic

41% of patients with long-COVID had moderate to severe sleep disturbances

C Pena-Orbea, B Lapin, Y Li, *et al.* **Sleep Disturbance Severity and Correlates in Post-acute Sequelae of COVID-19 (PASC).** *J GEN INTERN MED,* 4 April 2023

Inflammation is culprit behind long-COVID

- S1 protein readily passed through BBB
- Protein caused inflammation that started problems with:
  - Learning and memory
  - Accelerate effects of Alzheimer disease and cognitive impairments
- Virus can not cross the BBB without S1 protein
- Omicron passed the barrier most easily

Inflammation is culprit behind long COVID, study suggests. UW Medicine Newsroom. 24 Feb 2023
MA Erickson, AF Logsdon, EM Rhea, et al. **Blood-brain barrier penetration of non-replicating SARS-CoV-2 and S1 variants of concern induce neuroinflammation which is accentuated in a mouse model of Alzheimer's disease**. *Brain, Behavior, and Immunity*, March 2023;109:251-268

Dietary inflammation linked to worse outcomes in COVID-19

- Over 500,000 participants
- Results consistent both with protective effects of anti-inflammatory diets and chronic pro-inflammatory effect of high levels of adiposity

Zhao L, Wirth MD, Petermann-Rocha F, et al. **Diet-Related Inflammation Is Associated with Worse COVID-19 Outcomes in the UK Biobank Cohort.** *Nutrients,* 2023 Feb 9;15(4):884

Long-COVID

Findings:

- COVID-19 infection has more than **50 long-term effects**
- 228 of 348 participants had at least 1 symptom suggestive of vagus nerve dysfunction – diarrhea most frequent symptom
- Exercise produce exerkines – substances produced/generated with exercise (includes hormones and metabolites)
- Strength training essential – long-hauler has typical loss of muscle-mass

Mayca González, **Precision Health: A COVID-19 Professional Debate**. *7th International Congress of the Spanish Society of Precision Health*, May 2022

High BMI and female sex risk factors for long-COVID symptoms

Debski M, Tsampasian V, Haney S, et al. (2022) **Post-COVID-19 syndrome risk factors and further use of health services in East England**. *PLOS Glob Public Health* 2(11): e0001188

How long COVID exhausts the body

4 factors increase risk/immune system:

- High levels of viral RNA during infection
- Presence of certain autoantibodies
- Reactivation of Epstein-Barr virus
- Having type-2 diabetes

Long-COVID patients have:

- Disrupted immune systems
- Viral genetic material remain embedded in intestines and lymph nodes

New York Times, 19 Feb 2022

How long COVID exhausts the body (cont'd)

Circulatory system:

- Small fiber neuropathy – associated with dysautonomia
- Microscopic blood clots
- Increased cytokines – decreased usage of O_2

Brain:

- Cognitive impairment
- Reduction of amount of blood leading to chronic fatigue

New York Times, 19 Feb 2022

Long term neurologic outcomes of COVID-19

- 12 months following acute COVID-19 infection
- increased risk of array of neurologic sequelae:
  - Stroke
  - Cognitive/memory disorders
  - PNS disorders
  - Migraines
  - GBS
  - M/S disorders
  - Movement disorders
  - Mental health

Conclusion: There is increased risk of long-term neurologic disorders in people who had COVID-19

E Xu, Y Xie, Y, Z Al-Aly. **Long-term neurologic outcomes of COVID-19**. *Nat Med,* 22 Sept 2022

Post-acute COVID-19 syndrome

- Median time 351 days post-COVID-19
- Common persistent symptoms:
  - Fatigue – 82%
  - Brain fog – 67%
  - Headache – 60%
- Symptom exacerbation:
  - Physical exertion – 86%
  - Stress – 69%
  - Dehydration – 49%

Conclusion:

Persistent symptoms associated with PASC impact physical and cognitive function

L Tabacof, J Tosto-Mancuso, J Wood, et al. **Post-acute COVID-19 syndrome negatively impacts physical function, cognitive function, health-related quality of life and participation.** *American Journal of Physical Medicine & Rehabilitation,* October 20, 2021. doi: 10.1097/PHM.0000000000001910

Cognitive symptoms/COVID

Patients with long-COVID reported current cognitive issues:

- 78% - difficulty concentrating
- 69% - brain fog
- 67.5% - forgetfulness
- 59.5% - difficulty recalling a desired word
- 43.7% - saying an unintended word

Panyuan G, Alvaro BB, Sabine PY. et al. **COVCOG 2: Cognitive and Memory Deficits in Long COVID: A Second Publication From the COVID and Cognition Study**. *Front Aging Neurosci*, 17 March 2022;14

Thyroid dysfunction may linger a year after severe COVID-19

American Thyroid Association (ATA) 2022 Annual Meeting,
oral abstract 12, 21 Oct 2022

Many health outcomes of people who developed long COVID after mild acute SARs-CoV-2 infection resolved 1 year later

Mizrahi B, et al. **Long COVID outcomes at one year after mild SARS-CoV-2 infection: nationwide cohort study.** *BMJ* 2023;380:e072529

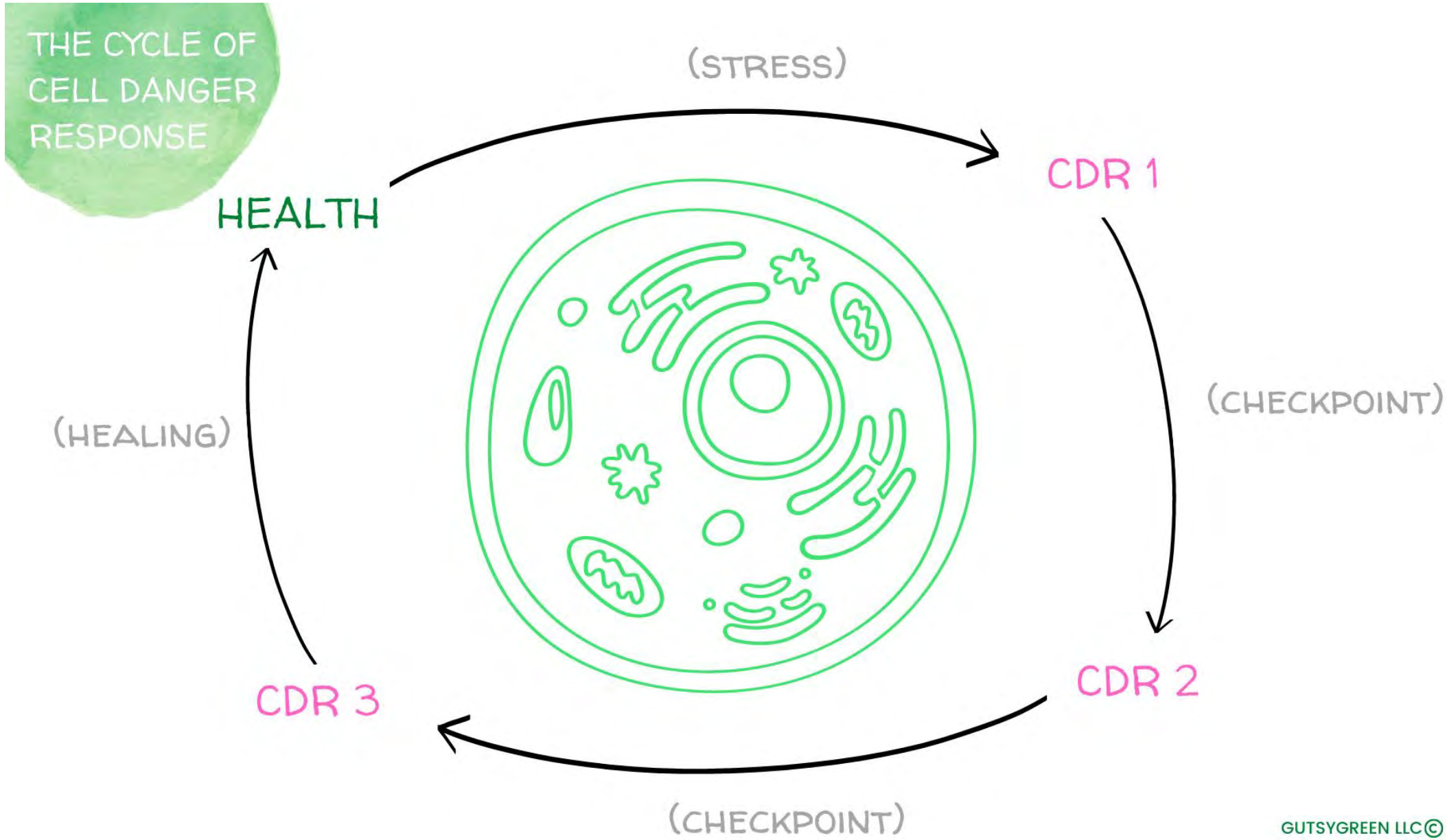
Biological changes of people post-COVID

- Disturbances in circulatory function:
  - Destruction of enzyme that is cellular receptor for the virus
- Ongoing state of inflammation in the body:
  - Immune system typically not returned to pre-COVID state
  - Abnormalities in T cell function
  - High rate of autoantibody function
- Mitochondrial distress/dysfunction
- Gut microbiome – maybe first:
  - Loss of diversity
  - Loss of anti-inflammatory organism
  - Increased level of inflammatory-reducing microbes
  - Increased leaky gut occurrence

Cell danger response (CDR)

- CDR – complex innate defense by our individual cells to danger/cellular threat
- Our cells must maintain certain level of energy for cellular homeostasis
- If drop in energy – our mitochondria senses as threat
- Results in mitochondria changing primary function from energy production to cell defense
- Switch called cell danger response

Naviaux RK. **Metabolic features of the cell danger response.** *Mitochondrion*. 2014 May;16:7-17



https://www.gutsygreen.com/blog/how-mitochondrial-dysfunction-and-cell-danger-response-lead-to-chronic-illness

Long COVID and Epstein-Barr Virus (EBV) reactivation

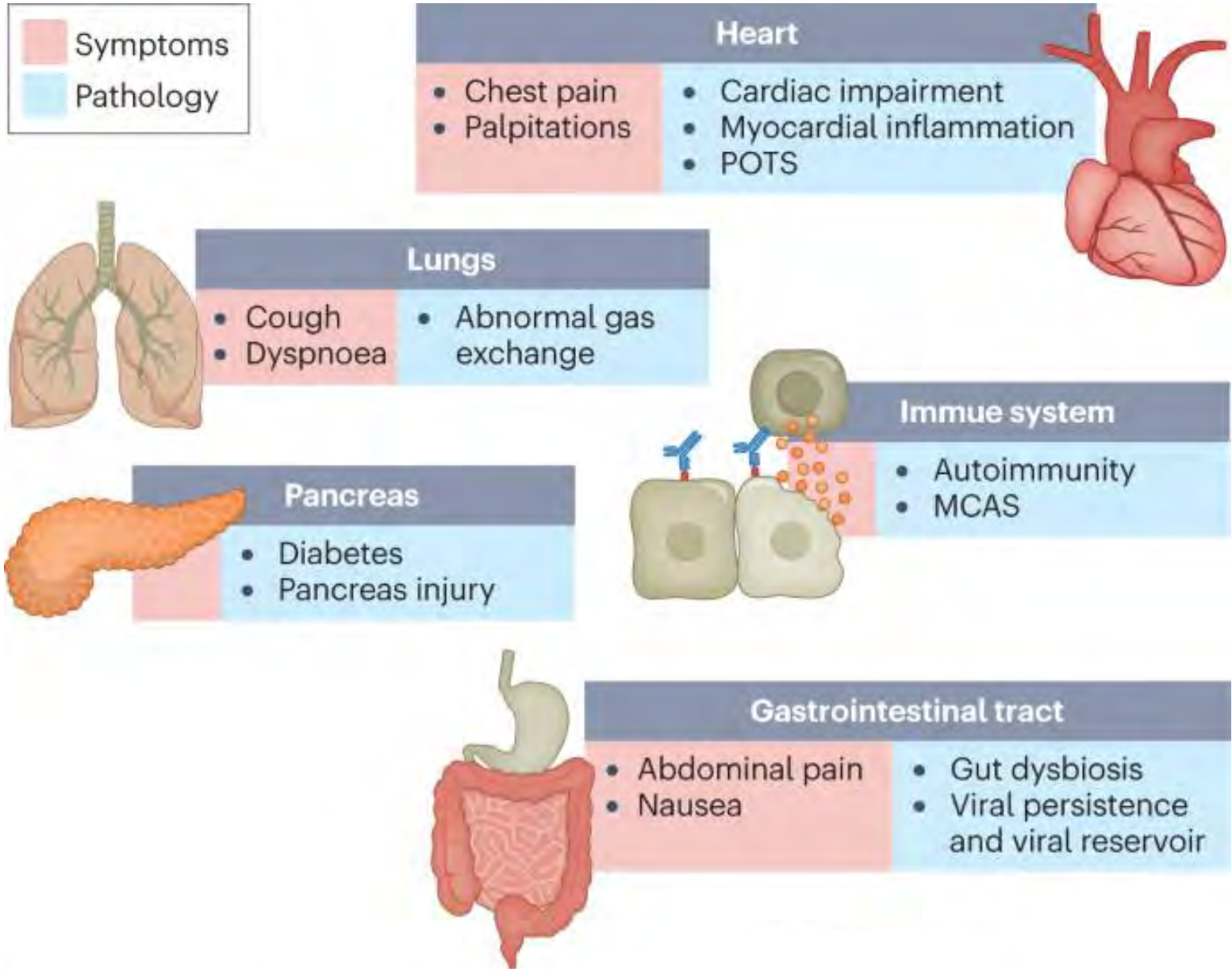
- 185 randomly selected COVID-19 patients
- 30.3% developed long-COVID
- Of those, 66.7% long COVID subjects versus 10% control subjects – positive for EBV reactivation
- Reactivation of EBV may occur early in COVID-19 infection cycle

Conclusion:

Many long COVID symptoms may not be direct result of SARs-CoV-2 virus but may be result of COVID-19 inflammation-induced EBV reactivation

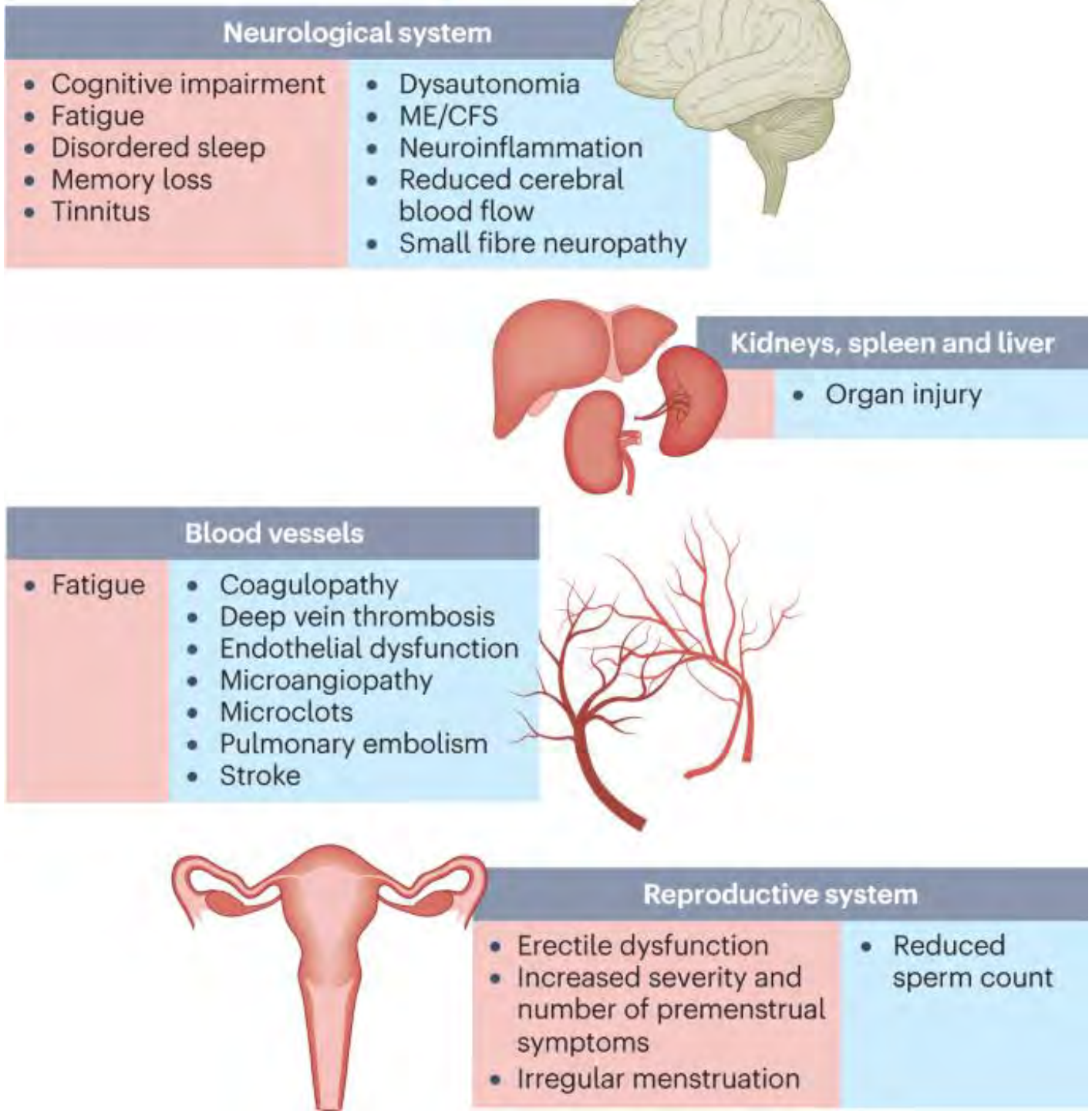
Gold JE, Okyay RA, Licht WE, Hurley DJ**. Investigation of Long COVID Prevalence and Its Relationship to Epstein-Barr Virus Reactivation**. *Pathogens*. 2021 Jun 17;10(6):763

Long COVID symptoms and the impacts on numerous organs with differing pathology



Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol*. 13 Jan 2023

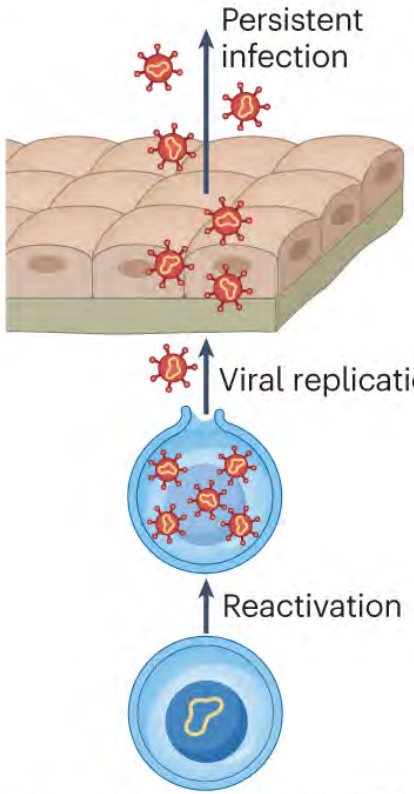
Long COVID symptoms and the impacts on numerous organs with differing pathology (cont'd)



Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol*. 13 Jan 2023

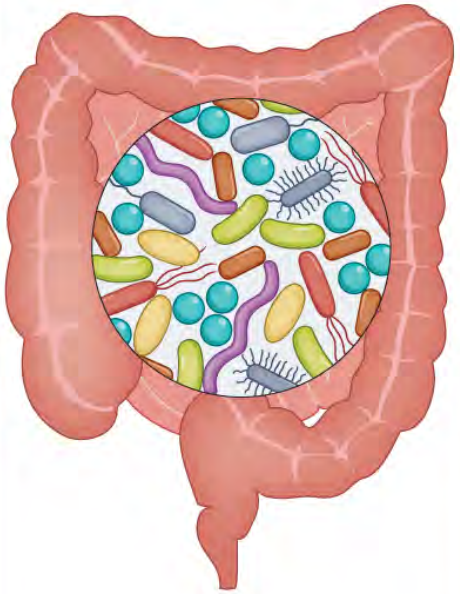
Hypothesized mechanisms of long COVID pathogenesis

Immune dysregulation



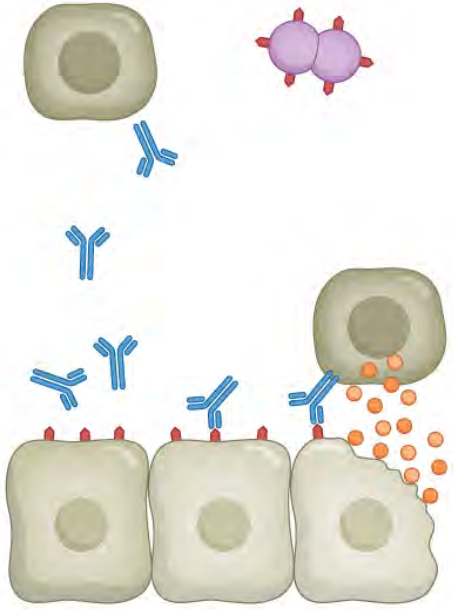
Immune dysregulation, with or without reactivation of underlying pathogens, including herpesviruses such as EBV and HHV-6

Microbiota dysbiosis



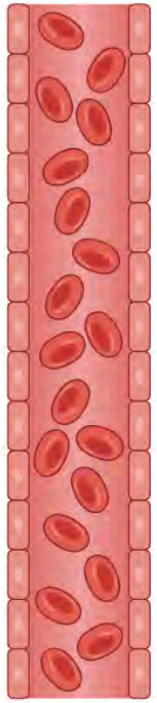
Impacts of SARS-CoV-2 on the microbiota and virome (including SARS-CoV-2 persistence)

Autoimmunity and immune priming



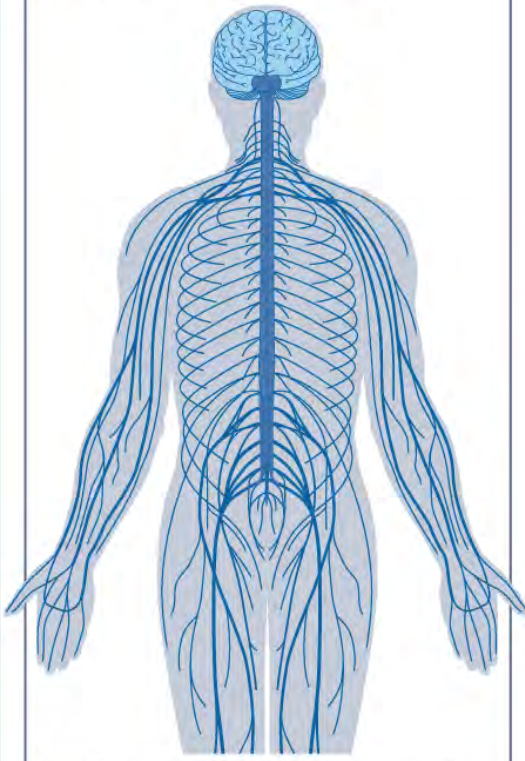
Autoimmunity and primed immune cells from molecular mimicry

Blood clotting and endothelial abnormalities



Microvascular blood clotting with endothelial dysfunction

Dysfunctional neurological signalling



Dysfunctional signalling in the brainstem and/or vagus nerve

Long COVID: major findings, mechanisms and recommendations. *Nat Rev Microbiol*. 13 Jan 2023

Do we have a better answer?

YES!