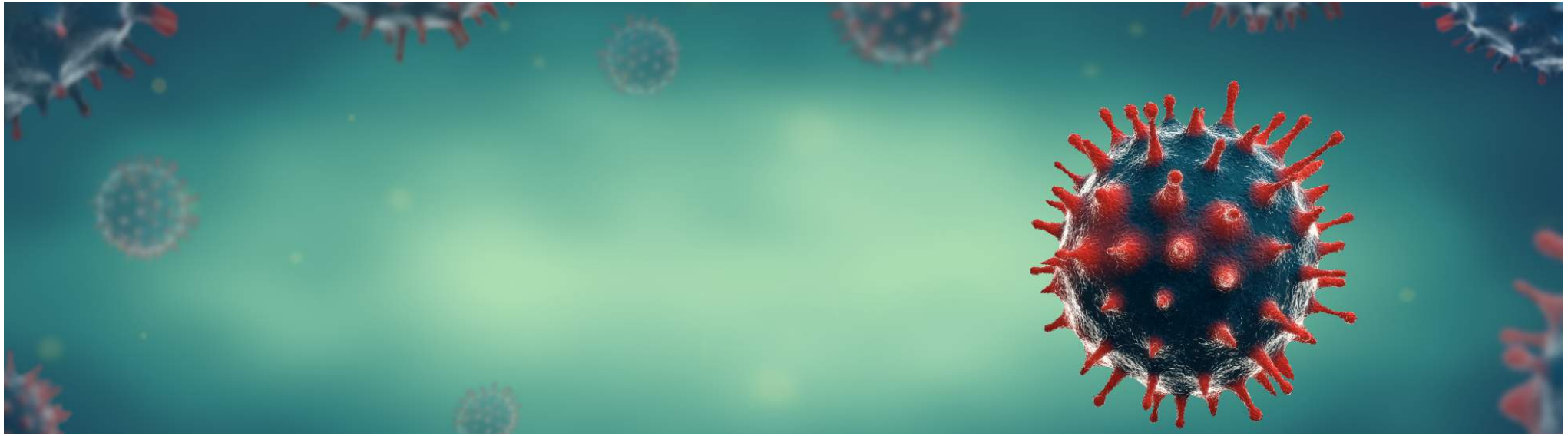
Modifiable Lifestyle Factors, Viral Infection
&
Immune Resilience

Testing and Assessment: Personalizing Interventions in Clinical Practice.

Robyn Puglia
IFMCP
DipION FdSc



Robyn Puglia

- Clinical Nutritionist and Certified Functional Medicine Practitioner
- The Applied Functional Medicine Mentoring Program
- VP Education, Cyrex Labs UK, EU and Ire
- Consultant Educator, Pure Encapsulations UK
- The Immunity Community

No financial conflicts to declare

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We are a human-microbe kibbutz

You can't treat a virus as an individual entity, separate from the immune system or the body in which it resides.

- Our virome is part of our microbiome, part of the ecosystem, so we need to consider it in that context.
- In chronic viral infection problems arise when a significantly imbalanced immune system such as we see with CFS or AID, isn't capable of mounting the appropriate responses to keep the virus/s in a symbiotic state of latency.
- So strategy needs to involve not just direct antiviral therapies, but also whole body, immune system and cell support, and it needs to be in place for as long as it takes for the immune system to be healthy enough to take over again.

In Clinical Practice

- We have to translate a lot of complex data into actionable, realistic interventions.
- How do we know when to apply common sense, general interventions?
- How do we know when to apply more specific, individual interventions?

In the age of personalized nutrition and lifestyle medicine – we must move beyond ‘protocol’ thinking.

First: Assess the patient

- Remember, **we are working with a person**. The person with the health condition or infection. Not just treating the infection or the downstream consequences of the infection.

- Taking a comprehensive history and populating the timeline, retelling the patient's story is STILL THE MOST IMPORTANT ASSESSMENT TO BEGIN WITH

For consideration

- Antecedent factors and conditions
- The immune terrain
- Lifestyle and nutrition

Assess:

- What is in this person's body or life, that is going to promote or exacerbate viral replication, mitochondrial dysfunction and aggressive inflammatory immune responses? (remove)
  - Oxidative stress
  - Inflammation
  - NF-kB
- What is missing from this person's body, or life, that is required for healthy immune responses and defenses? (replace)
  - Vitamins
  - Minerals
- Post Viral – What tissues have been damaged or organ/physiological systems disrupted that need to be repaired?

Investigating Antecedents

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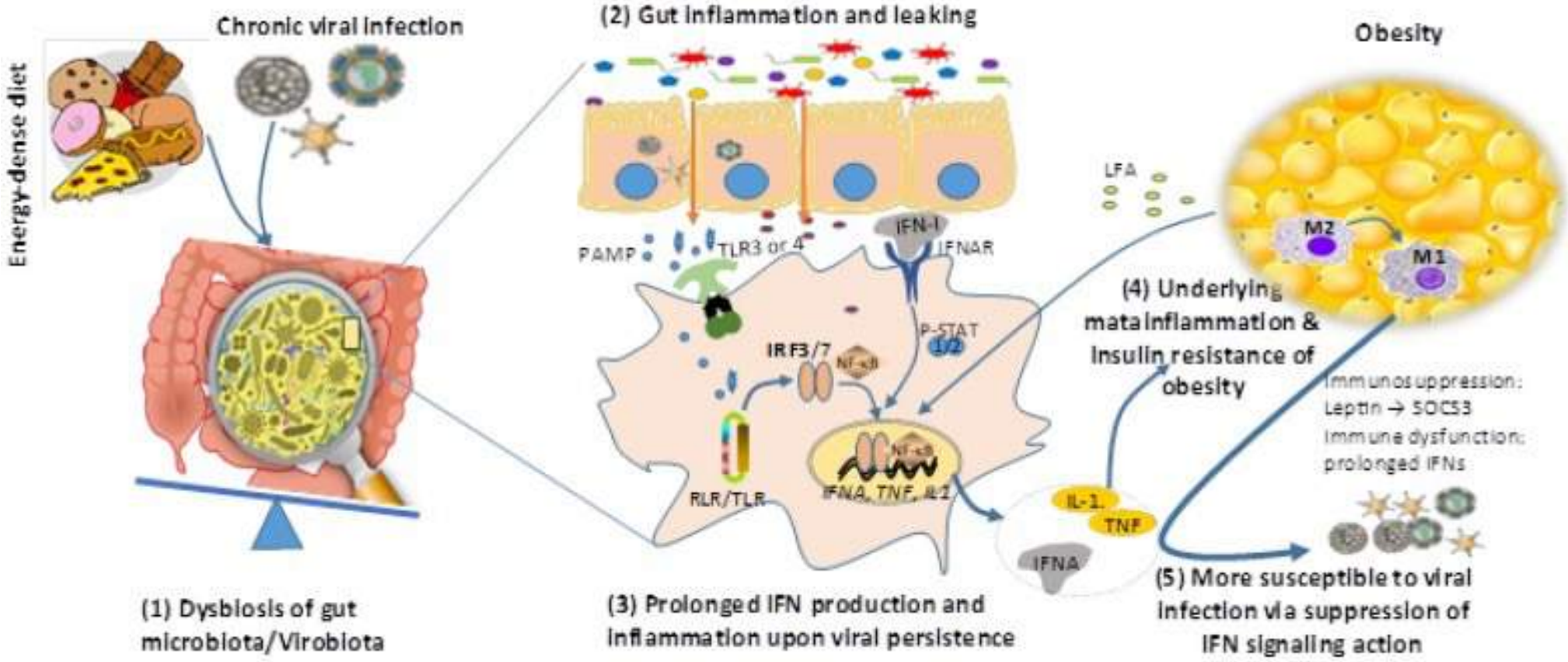
Categories of Vulnerabilty

Excess Inflammation	Organ Dysfunction	Compromised Immunity
CVD	Asthma	Transplant
Type 1 Diabetes	CVD	HIV
Type 2 Diabetes	Hypertension	Immune Deficiencies
Hypertension	Pregnancy	Neurological Conditions
BMI >30	Smoking	Pediatrics
Asthma	Genetic Metabolic Disorders	Immunosuppressive Medications
Smoking	Liver disease	
	COPD	
	Chronic Kidney Disease	
	Cancer	
	Type 1 Diabetes	
	Immune Deficiencies	

Obesity and Viral Infection

- Several viruses or virus-like agents including members of adenoviridae, herpesviridae, slow virus (prion), and hepatitides, have been associated with obesity; meanwhile obese patients are shown to be more susceptible to viral infections such as during influenza and dengue epidemics.
- Antiviral interferons (IFNs), as key immune regulators against viral infections and in autoimmunity, emerge to be a pivotal player in the regulation of adipogenesis.
- However, the prolonged IFN responses during persistent viral infections and obesogenesis comprise reciprocal causality between virus susceptibility and obesity.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6920831/


Energy-dense diet
Chronic viral infection
(1) Dysbiosis of gut microbiota/Virobiota
(2) Gut inflammation and leaking
PAMP
TLR3 or 4
IFN-I
IFNAR
P-STAT 1/2
IRF3/7
NF-κB
RLR/TLR
IFNA, TNF, IL2
(3) Prolonged IFN production and inflammation upon viral persistence
LFA
IL-1
TNF
IFNA
(4) Underlying metainflammation & insulin resistance of obesity
Obesity
M2
M1
Immunosuppression: Leptin → SOCS3
Immune dysfunction: prolonged IFNs
(5) More susceptible to viral infection via suppression of IFN signaling action

Glycaemic Control and Viral Illness

- There is a reciprocal, detrimental interaction between the immune and endocrine system in the context of T2D.
- The recent pandemic of COVID-19 has made abundantly clear that Type 2 diabetes (T2D) increases the risk of more frequent and more severe viral infections.
- At the same time, pro-inflammatory cytokines of an anti-viral Type-I profile promote insulin resistance and form a risk factor for development of T2D.

Endocrine influence on Immunity

- Metabolism of immune cells is mostly regulated by cytokines but they are not exempt from endocrine control.
- Many hormone receptors share intracellular signaling components with those of immune receptors, indicating an overlap in function.

https://doi.org/10.1186/s13045-016-0329-3

- Recently, insulin itself was identified as a molecule that can directly regulate immune cell function, most notably of T cells
- Both CD4 and CD8 T cells express the insulin receptor on their cell surface upon activation
- **Loss of insulin receptor expression on T cells impairs proliferation and cytokine production of anti-viral T-cells**

Immunity, 49 (164–77) (2018), Article e6

Type 2 diabetes and viral infection; cause and effect of disease

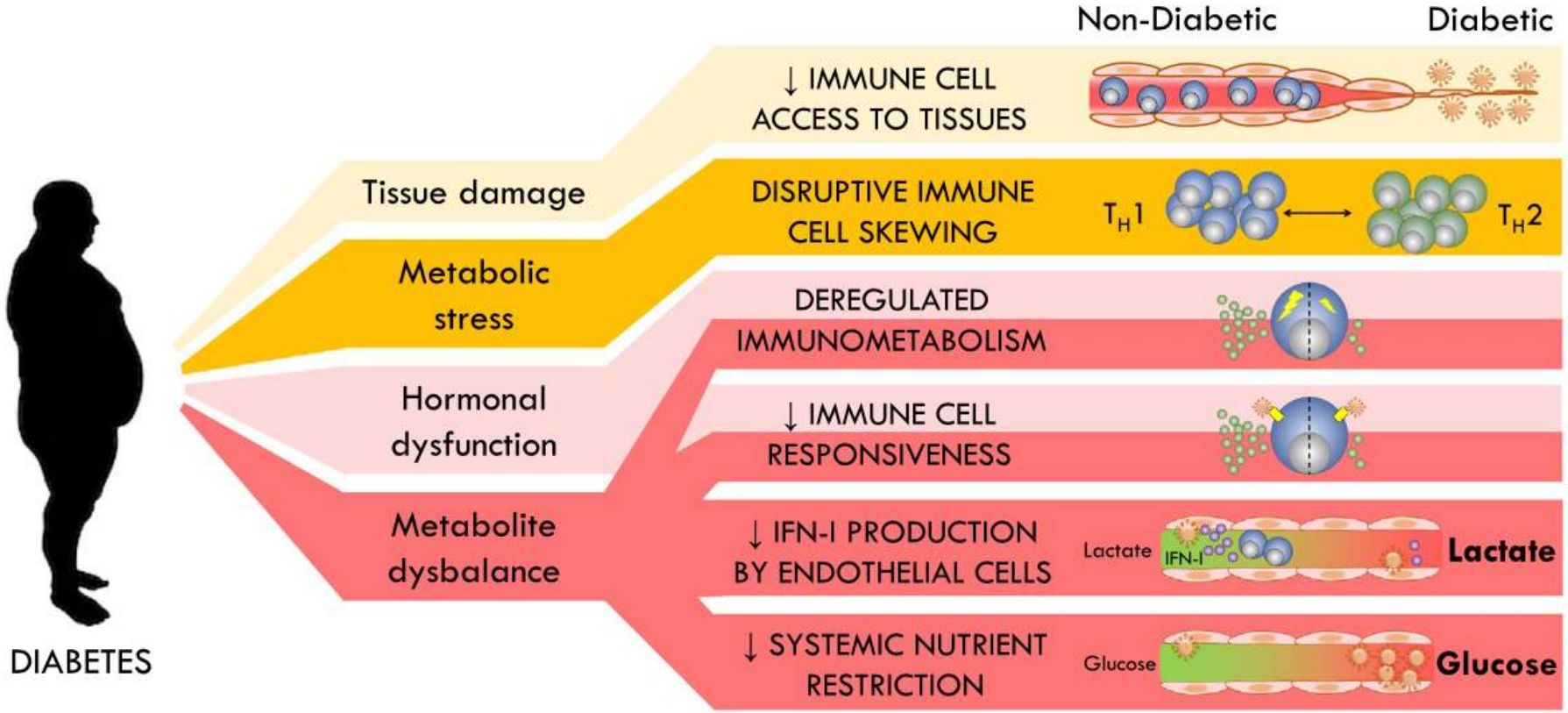


Fig. 1 – Negative impacts of T2D on immunological control of viral infection.

https://doi.org/10.1016/j.diabres.2020.108637

Chronic viral infection as a risk factor for T2D

- Infection is a risk factor for development of T2D, mostly shown for chronic viruses.
- A population-based matched case-control cohort study in Korea selected 576 patients infected with cytomegalovirus (CMV), but withoutT2D and 2880 matched controls without either condition and followed them for 5 years for development of new onsetT2D.
- the case group had a much higher frequency of new-onset T2D (5.6% vs. 2.2% $p < 0.001$).
- Patients with refractory disease had a significantly higher incidence rate (OR 4.01 95% CI 1.76–7.69) than people with non-refractory disease (OR 1.77 95% CI 1.07–2.82) or with non-infected controls (reference population).

Biochem Biophys Res Commun; 355 (2007), pp. 883-888

Continuous Glucose Monitoring

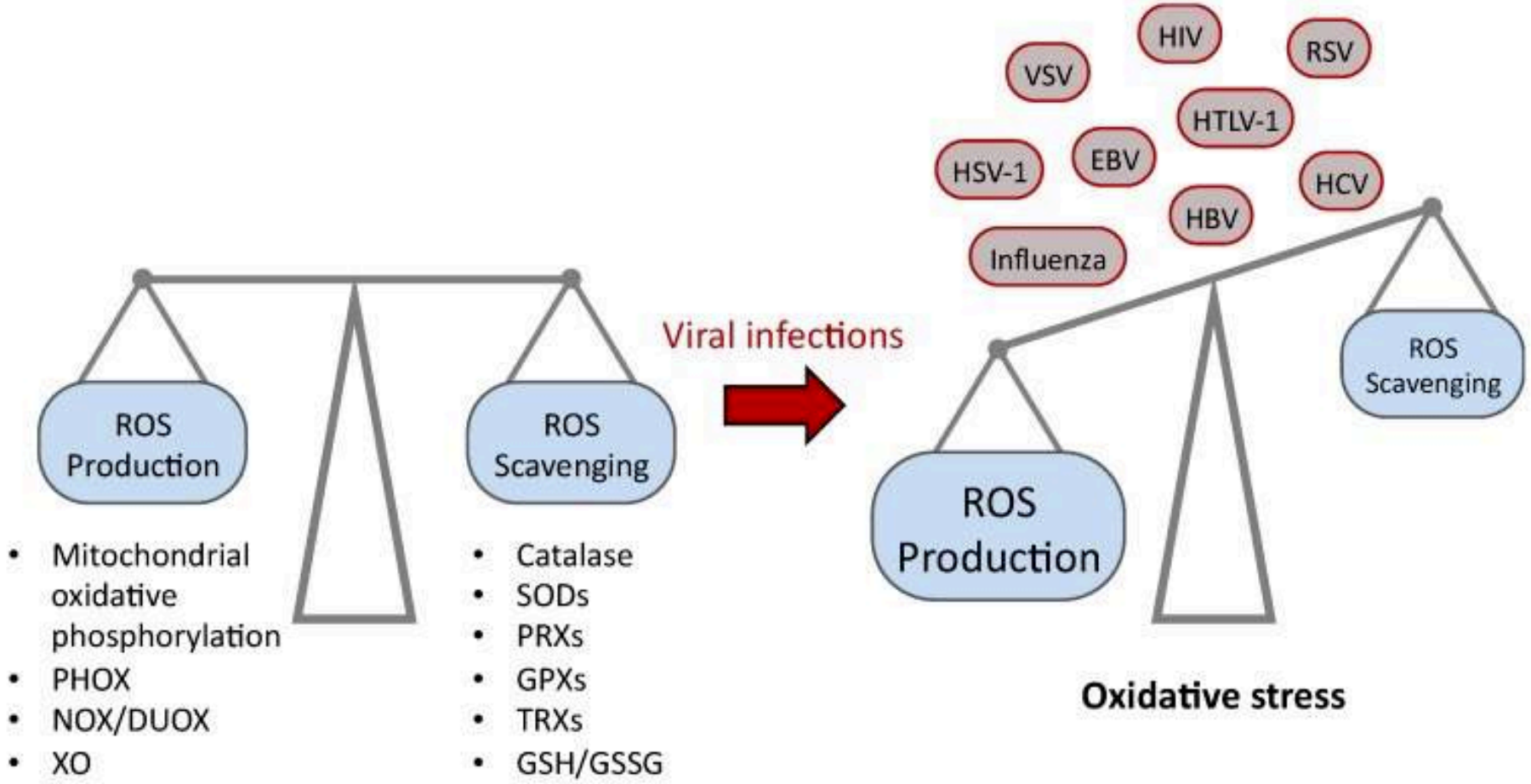
- Freestyle Libre
- Dexcom

*Reportedly not as reliable as fingerprick testing for levels below 3mmol/L and above 10mmol/L

Mitochondrial Function

- Fatigue is a hallmark not just of viral infection, but also post-viral syndrome and post-viral ME/CFS
- Viral infection creates a significant amount of oxidative stress, which can uncouple mitochondria and affect ATP production.
- But mitochondria themselves have an anti-viral function.
- Mitochondria work at level of innate immune system after recognition of PAMP's (pathogens) by Toll Like Receptors to activate genes to make proteins to destroy viruses.
- Mitochondrial Antiviral Signaling (MAVS) Protein **coordinates activation of interferon** and autophagy.

Understanding Inflammatory Responses in the Manifestation of Prothrombotic Phenotypes. Front Cell Dev Biol. 2020;8:73.



Nutrients. 2019 Sep; 11(9): 2101.
Published online 2019 Sep 4. doi: 10.3390/nu11092101
PMCID: PMC6769590
PMID: 31487871

Selenium, Selenoproteins and Viral Infection

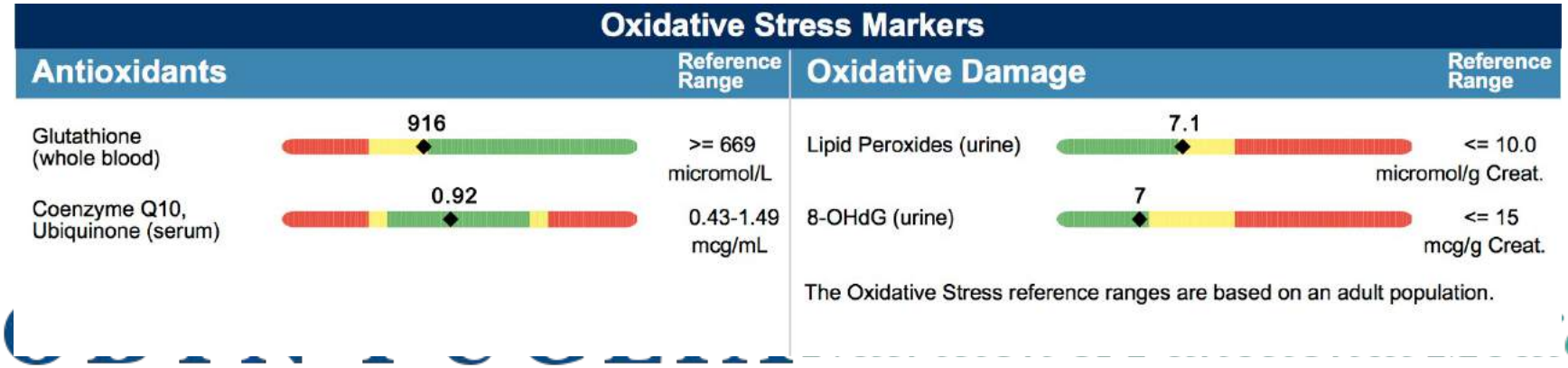
Genova Diagnostics Oxidative Stress 2.0 Blood

Analyte List
Cysteine
Cystine
Glutathione
Glutathione Peroxidase
Lipid Peroxides - Blood
Sulfate
Superoxide Dismutase (SOD)
Total Antioxidant Capacity (TAC)

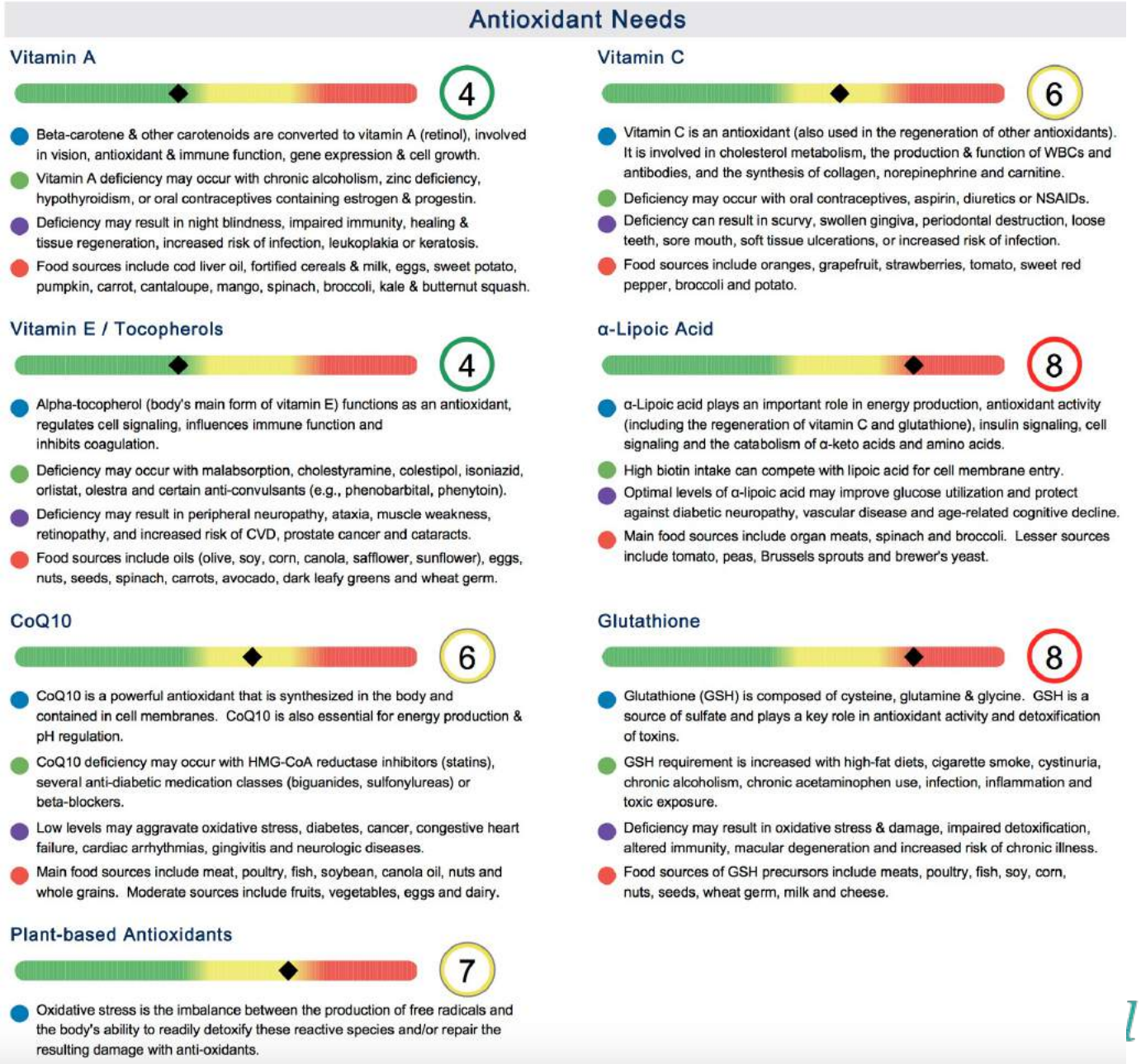
Genova Diagnostics
Oxidative Stress 2.0 Urine

Analyte List
8-OHdG
Lipid Peroxides

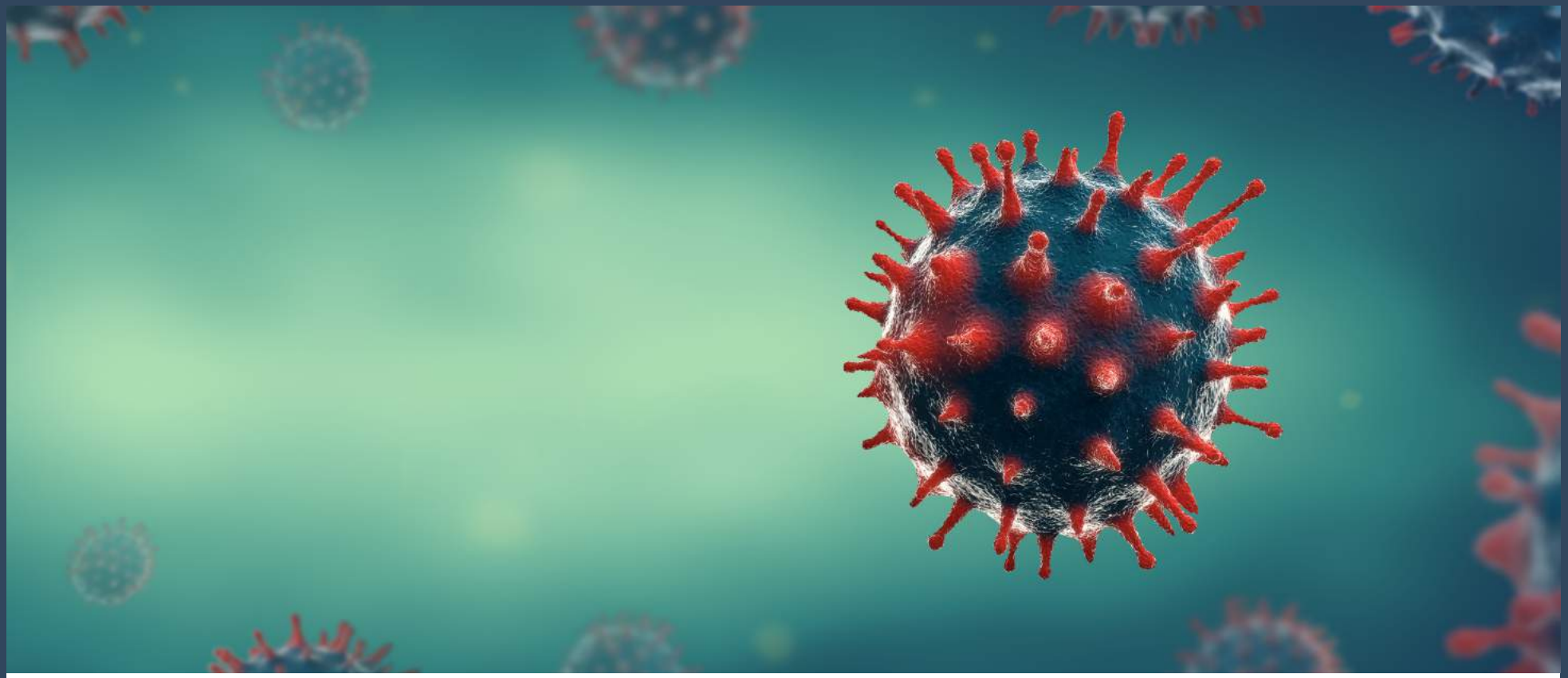
Genova Diagnostics
Oxidative Stress NutrEval



Genova Diagnostics NutrEval



ROB l Medicine



Understanding the Immune System

Consider history

- C-section
- Not breast fed or use of formula / Dx with CMPA in infancy and reintroduced in later childhood
- Hx of infections – Repeated childhood infections (ear, chest, tonsilitis etc), URIs, Sinus, UTIs etc.
- Atopy
- Fatigue
- Poor recovery from common infections
- Glandular Fever or unexplained viral illness
- Mould exposure
- Food reactivity
- First or second hand smoke exposure
- Autoimmune Dx or AID Mother
- Trauma / ACES

Sequence of Immune System Involvement in Viral Infection

- Pre-Exposure: Innate Immune System
- At Exposure: Mucosal Immune System
- Establishment of infection: Innate and mucosal
- Finally, Adaptive Immunity

Cell Host Microbe. 2016 Feb 10. 19(2):159-168. Janeway C

Mucosal Immune System

- The physical barrier and components of the immune system of the mucosa (especially secretory IgA – sIgA) prevent and/or limit active binding and infection by pathogens.
    - Chronic stress and increased cortisol lead to decrease in mucous production and decrease in sIgA production

Inflammation and Mucosal Immunity

Environmental

Environmental factors influencing inflammatory cascades include:

- Stress
- Gut dysbiosis
- Infections
- Dietary proteins
- Chemical toxicity

Medical History

Risk factors for mucosal immune dysregulation include:

- Smoking
- Alcoholism
- Chemotherapy and head/neck radiation
- Protein-energy malnutrition (PEM)

Cyrex Array 14 – Mucosal Immune Reactivity Screen

- Total Secretory IgA
- Lipopolysaccharides
- Occludin/Zonulin
- Actomyosin
- ASCA/ANCA
- Calprotectin
- Native + Deamidated Alpha-Gliadin-33-mer
- Gamma-Gliadin-15-mer
- Glutenin-21-mer
- Gluteomorphin
- Wheat Germ Agglutinin
- Transglutaminase-2
- Egg
- Soy
- Corn
- Alpha-Casein + Beta-Casein
- Casomorphin
- Aflatoxin
- Bisphenol-A
- Mercury
- Mixed Heavy Metals
- Rotavirus
- Myelin Basic Protein
- Blood-Brain Barrier Protein
- Immune Complex

Supporting Mucosal Immune System

Increased immune activity = increased metabolic requirements and energy needs

- Requires availability of substrates and molecular/ regulatory co -factors
- Vitamins = A, B6, B12, C, D, E, Folate
- Trace elements = Fe, Zn, Cu, and Selenium
- Improvement of gut microbiome
- Improve microbiome of respiratory tract:
  - S. salivarius K12

Di Pierro F. A possible probiotic approach to improve oral and lung microbiotas and raise defenses against SARS-CoV-2.
Minerva Med. 2020;111-281-283. doe: 10.23736/S0026-4806.20.06570-2

Innate Immune System Modifiers

- Improve Natural Killer Cell Function
  - Vitamin C
  - Vitamin D (test)
  - N-Acetyl Cysteine (NAC) (pyroglutamate , test oxidative stress)
  - Medicinal mushrooms + ß-glucans (test immune tolerance if Px has AID)

doi:10.1093/ecam/neh014

- Modulate Dendritic Cell Function
  - Vitamin A (Fat Soluble Vitamins, Oxidative Stress)
  - Vitamin D (test)
  - N-Acetyl Cysteine (NAC) (pyroglutamate, oxidative stress)
  - Astragalus
  - Elderberry

Blood chemistry markers for assessing immunity

- WBC
- Neutrophils
- Monocytes
- Basophils
- Vitamin D
- CRP
- ESR
- HBA1C and Fasting Glucose

Table to track changes

		Normal range	27 Feb 2018	4 Feb 2019	3 Jun 2019	4 Feb 2019	28 Jan 2020	4 Mar 21	8 Mar 21	10 Mar 21	25 Mar 21	12 Apr 21	14 Apr 21	15 Apr 21	28 Apr 21
Plasma viscosity	Plasma viscosity (XE2pd)	[1.5 - 1.72]									1.64 mPa.s				
Full blood count	Platelet count - observation (42P..)	[140.0 - 400.0]			266			233	261		268 10*9/L		276 10*9/L		288
Full blood count	Mean platelet volume	7.5 - 12													11.2
Full blood count	Neutrophil count (42J..)	2 - 7			3.62			4.91	5.09		3.55 10*9/L		3.08 10*9/L		2.03
Full blood count	Lymphocyte count (42M..)	1 - 3			2.61			0.54	2.07		2.92 10*9/L		2.65 10*9/L		2.33
Full blood count	Monocyte count - observation (42N..)	[0.2 - 0.8]			0.35			0.47	0.35		0.54 10*9/L		0.43 10*9/L		0.33
Full blood count	Eosinophil count - observation (42K..) (deals with parasites or allergy)	[0.1 - 0.4]			0.33			0.14	0.29		0.35 10*9/L		0.27 10*9/L		0.35
Full blood count	Basophil count (42L..)	[0.02 - 0.1]			0.05			0.04	0.04		0.07 10*9/L		0.05 10*9/L		0.05
Full blood count	Haematocrit (X76tb) (relates to the supply of red blood cells)	[0.37 - 0.47]			0.376			0.352	0.378		0.380 1/1		0.365 1/1		0.404
Full blood count	Red blood cell count (426..)	[3.8 - 5.8]			3.97			3.70	3.99		3.97 10*12/L		3.93 10*12/L		4.25
Full blood count	Haemoglobin concentration (Xa96v)	[115.0 - 165.0]			125			122	130		129 g/L		127 g/L		133
Full blood count	MCHC	288 - 352													329
Full blood count	RDW	12.2 - 16.1													11.7
Full blood count	Mean cell volume (42A..)	[80.0 - 100.0]			94.7			94.9	94.6		95.9 fL		92.9 fL		95.1
Full blood count	Mean cell haemoglobin level (XE2pb (protein that carries iron around)	[27.0 - 32.0]			31.6			32.9	32.5		32.4 pg		32.2 pg		31.3
Full blood count	Total white blood count (XaldY)	[4 - 10] healthy is between 5 and 6			7.0			6.1	7.8		7.4 10*9/L		6.5 10*9/L		5.1
LFT/Bone	Serum total protein level (XE2e9)	[60.0 - 80.0]						64	72	73	69 g/L		72 g/L		
LFT/Bone	Serum albumin level (XE2eA)	[35.0 - 50.0]						38	41	43	39 g/L		42 g/L		48
LFT/Bone	Serum alkaline phosphatase level (XE2px)	[30.0 - 130.0]						56	64	69	66 u/L		60 u/L		57
LFT	Serum alanine aminotransferase level (XaLJx)	[< 35.0]						14	15	24			22 u/L		23
LFT	Serum total bilirubin level (XaERu)	[< 21.0]						Less than 3	4	Less than 3			4 umol/L		7
Urea & Electrolytes/ Creatinine	Serum sodium level (XE2q0)	[133.0 - 146.0]		141	143		143	138	142	140			140 mmol/L		142

(Human) Herpes Virus Family

- HSV 1 + 2
- EBV
- CMV
- HHV6
- Varicella Zoster

T-cell testing (ELIspot, LTT etc)

- The T cell system plays an essential role in infections, allergic reactions, tumor and transplant rejection, as well as autoimmune diseases.
- Cytokine ELISPOT assays have emerged as a powerful tool for the detection of antigen-specific T cells in blood.
- The goal of most ELISPOT experiments is to identify positive T-cell responses as defined by a significantly elevated spot count in antigen-stimulated wells over the nonstimulated medium-control or negative-control antigen.

- ELISPOT assays have an unsurpassed sensitivity in detecting low frequency antigen-specific T cells that secrete effector molecules, including granzyme and perforin. They provide robust, highly reproducible data

Immune Monitoring for CMV in Transplantation

- Immune monitoring to determine when and how the recovery of cytomegalovirus (CMV)-specific T-cells occurs post-transplantation may help clinicians to risk stratify individuals at risk of complications from CMV
- Post-transplant CMV immune monitoring can guide (shorten or prolong) the duration of antiviral prophylaxis, identify recipients at risk of post-prophylaxis CMV disease, and predict recurrent CMV reactivation
- Quantiferon-CMV and the CMV ELISPOT

Curr Infect Dis Rep. 2018 Mar 14;20(4):4.
doi: 10.1007/s11908-018-0610-4.
Immune Monitoring for CMV in Transplantation

Epstein Barr Virus Testing

- EBV – IgG
- EBV – IgM
- EBV Nuclear Antigen – IgG, IgM
- EBV Viral Capsid Antigen- IgG, IgM
- EBV Early Antigen - IgG

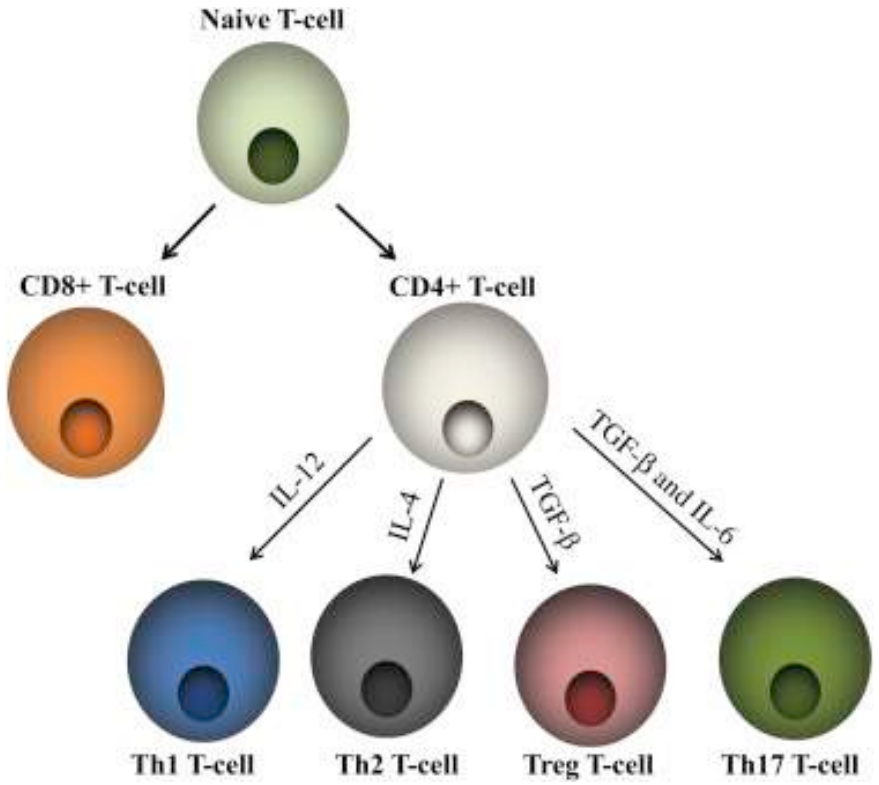
- Elevated monocytes on blood chem
- ITT or Ellispot
- DNA in saliva

Balfour HH Jr, Dunmire SK, Hogquist KA.
Infectious mononucleosis.
Clin Transl Immunology. 2015;4:e33

Mentzer AJ, et al.
Identification of host-pathogen-disease relationships using a scalable Multiplex Serology platform in UK Biobank.
medRxiv. 2019;19004960.

EBV ELIspot

- **CD8+ cells** are cytotoxic **cells** that induce apoptosis in **cells**, which presents the cognate antigen on MHC class I. Although crucial for responses against intracellular viruses and bacteria
- the ELISPOT assay can be applied to the analysis of CD8(+) responses to EBV antigens in blood cells…
  - **(Blood. 2000;95:241-248)**



ELIspot viral panel results

Cytomegalovirus Elispot

Cytomegalovirus Elispot	**112**	**+**	SI	< 2	

Test result cellular immunity:

Specific interferon-gamma, targeting the cytomegalovirus, confirmed hereby cellular involvement of T-lymphocytes.

Epstein-Barr-Virus Elispot

Epstein-Barr-Virus (lytic) Elispot	1		SI	< 2	

cellular immunity - LY:

By measuring specific targeted interferon-gamma against lytic or, respectively, late phase antigens of EBV, no reactive T-lymphocytes could have been traced.

Epstein-Barr-Virus (latent) Elispot	**85**	**+**	SI	< 2	

cellular immunity - LA:

Specific interferon-gamma, targeting lytic or, respectively, latent phase antigens of EBV, confirmed hereby cellular involvement of T-lymphocytes.

Regenerus Labs

- Virus Activity Profile (4M4527)
  - DNA / RNA in saliva
  - HSV1, HSV2, HHV6, CMV, VZV, EBV
- Virus Profile ITT (ELIspot) (4M6640)
  - EBV, CMV, VZV, HSV
- EBV ITT (4M5350)
- AONM / Armin Labs
  - EBV ELISpot Lytic and Latent

Stress inhibits formation of new lymphocytes

- During T cell development, immature thymocytes progress from double negative (for the CD4 and CD8 T cell markers) to double positive cells ($CD4^+CD8^+$) which undergo positive selection (only thymocytes that bind MHC complexed with self-antigen survive) and negative selection (against cells that interact too strongly with self-antigen) to mature into either $CD4^+$ or $CD8^+$ single positive cells; the T cell repertoire.
- Double positive cells, the majority of the thymocyte population, are highly sensitive to glucocorticoid-induced apoptosis

- Glucocorticoids halt formation of new lymphocytes in the thymus
- Most of thymic tissue is made up of these new cells, ready to be secreted into the bloodstream

Expression of the glucocorticoid receptor from the 1A promoter correlates with T lymphocyte sensitivity to glucocorticoid-induced cell death.
J Immunol. 2004 Sep 15; 173(6):3816-24.

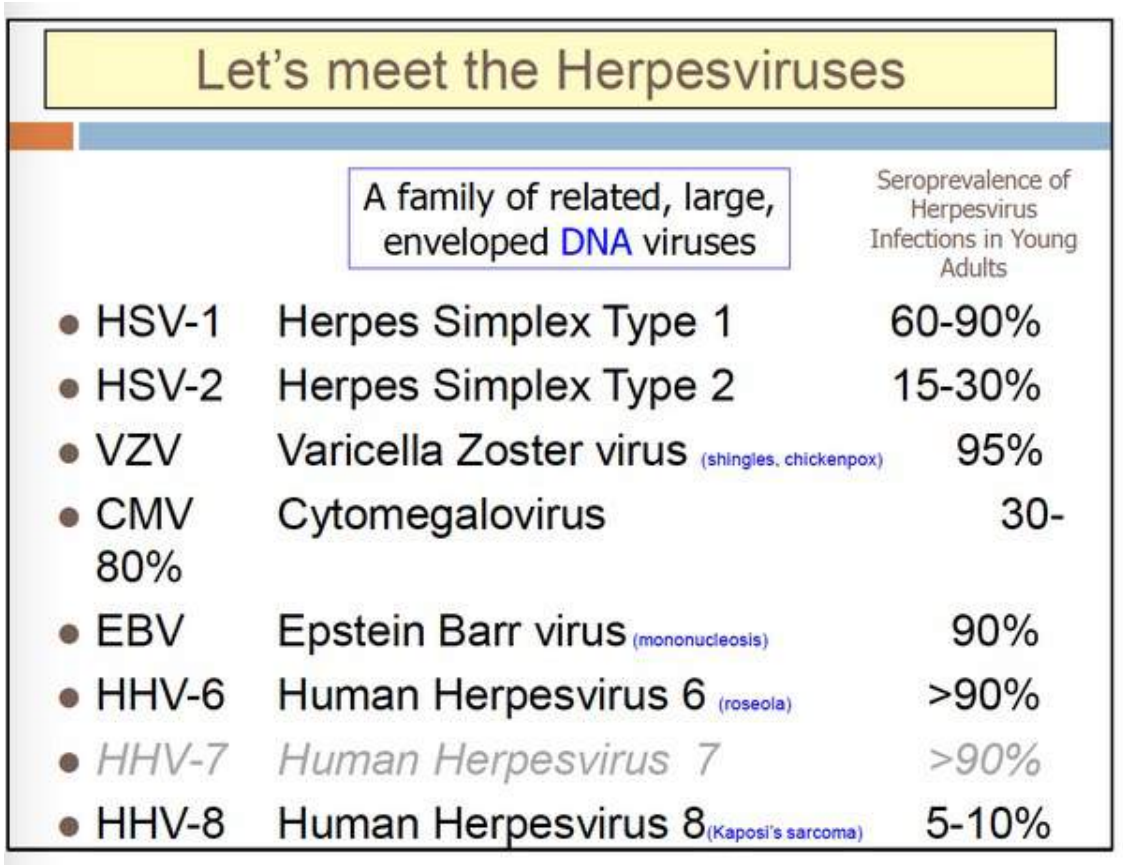
- Herpes virus DNA (including EBV and VZV) contains a stretch that is sensitive to elevated glucocorticoid levels.

- Same stretch of DNA activates the genes that involved in reactivation

Human Herpesviruses: Biology, Therapy, and Immunoprophylaxis.
Arvin A, Campadelli-Fiume G, Mocarski E, et al., editors.
Cambridge: Cambridge University Press; 2007.

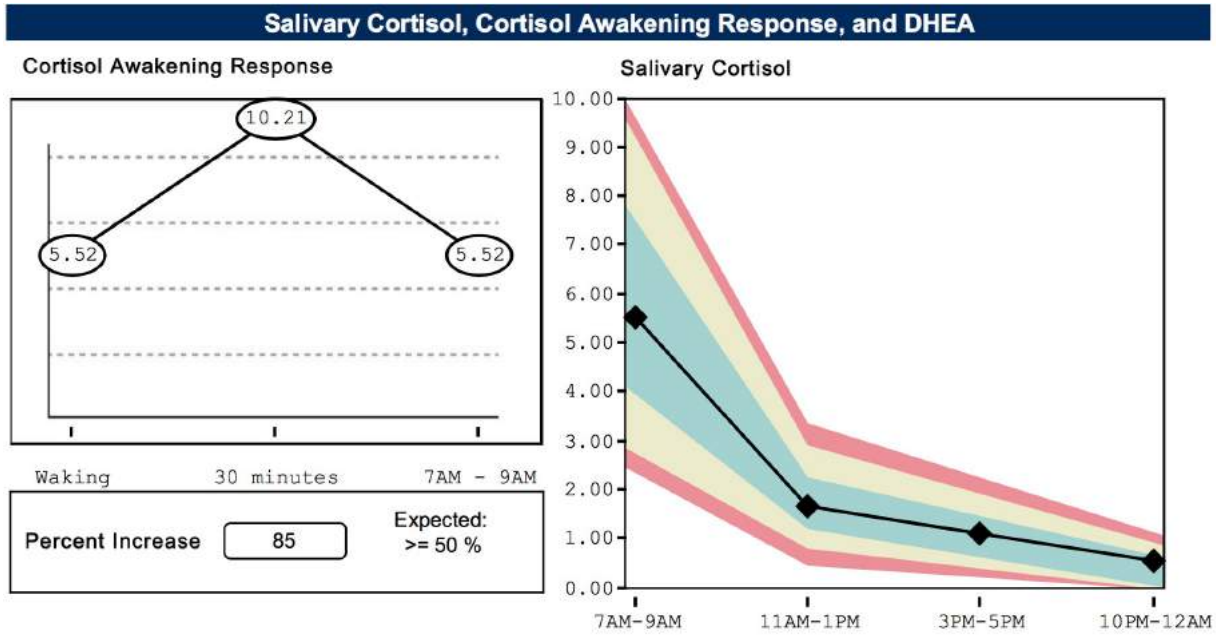
Viruses are a stressor

- Herpes virus family also:
- Stimulates the hypothalamus to release CRH which releases ACTH
  - = elevated glucocorticoids



Cortisol Awakening Response

- The cortisol awakening response (CAR) is a distinct facet of the circadian cortisol rhythm, an increase of cortisol within the first hour after awakening that is separate from the cortisol increase during the second half of the night
- Within about the first 30 minutes of a new stress reaction, immune defense is actually enhanced.
- Primarily mediated by adrenaline.
- Generic antibodies are released into saliva (innate immunity)
- Immune cells are rushed into the circulation
- Circulating lymphocytes are better at responding to immune messengers



ROBY

nal Medicine

Applying Personalized Lifestyle Interventions

ROBYN PUGLIA *Nutrition & Functional Medicine*

Nutrition is critical

The immune system puts a major drain on systemic resources and can use up to 30% of all the body's nutrients in circulation upon infection.

Am J Hum Biol, 22 (2010), pp. 546-556

Treat the patient.

- If someone has severe dysbiosis and small intestinal fermentation and very slow phase one hepatic biotransformation and you put them on a diet full of brassicas, onions, garlic and fibre because those foods support liver health, glutathione, SCFA and immune health– are they going to feel better and get better?

Critical Lifestyle Interventions

- SLEEP – (number 1 anti-viral) Tracking for duration, REM to Deep sleep cycles.
- Stress - (number 1 viral reactivator)
- Movement – Avoid sedentary and overtraining behaviour
- Nutrition

Targeted Therapeutic Nutrition Provides

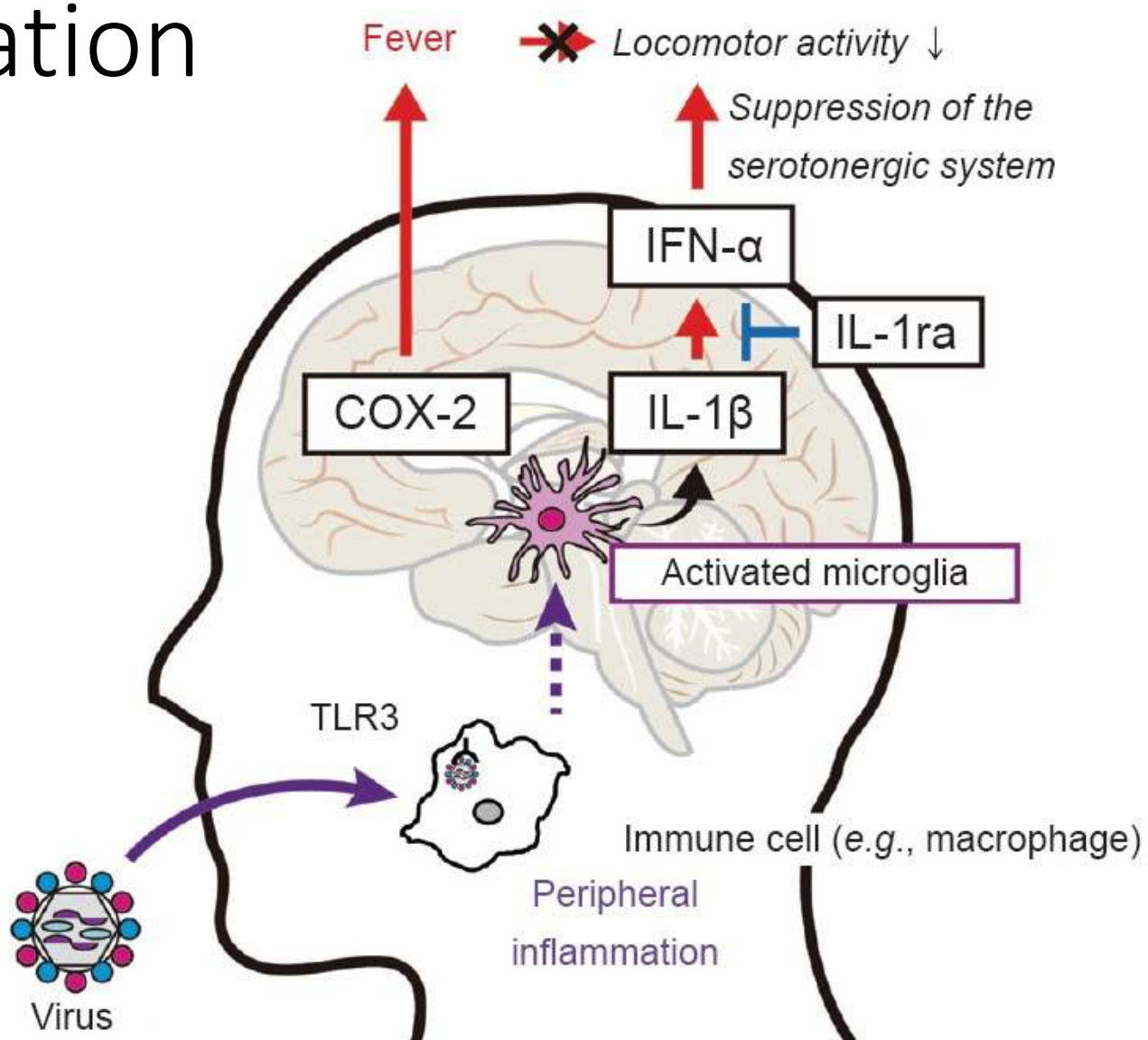
- Antioxidants and Glutathione Support
- Fibre for SCFA
- Nutrients to modulate and support mitochondrial functional, Mucosal Immunity, Innate Immunity and Adaptive Immunity
- Fat soluble nutrients (immune, antiviral)
- Minerals (immune, antiviral)
- Macronutrients for mitochondrial function, blood glucose regulation and stress management
- Anti-inflammatory nutrients

Post Viral Fatigue and Neurological Inflammation

- The fatigue sensation is thought to be one of the signals for the body to suppress physical activity in order to regain health.
- The mechanism of induction of the fatigue sensation following viral infection has not been well understood.
- **Although fatigue was once thought to be caused by fever, our recent study with an animal model of viral infection demonstrated that the fatigue sensation is caused not by fever, but rather, by neuroinflammation of brain tissue**

PLoS One. 2014;9(3):e90950. Published 2014 Mar 12.
doi:10.1371/journal.pone.0090950

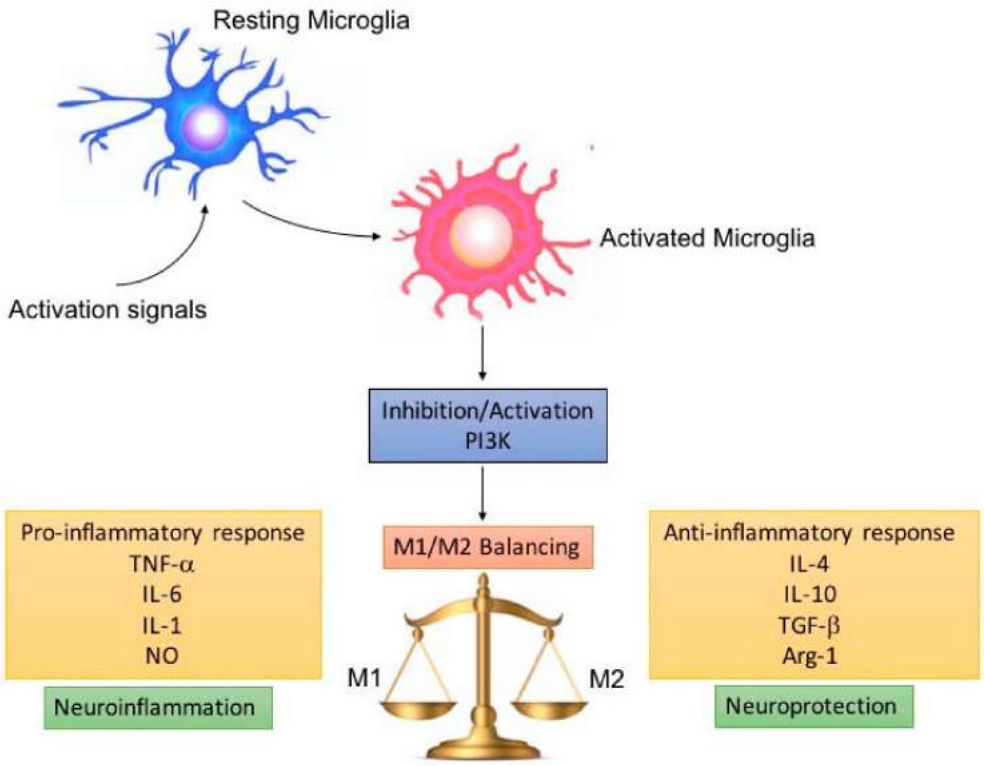
Viral Induced Neurological Inflammation



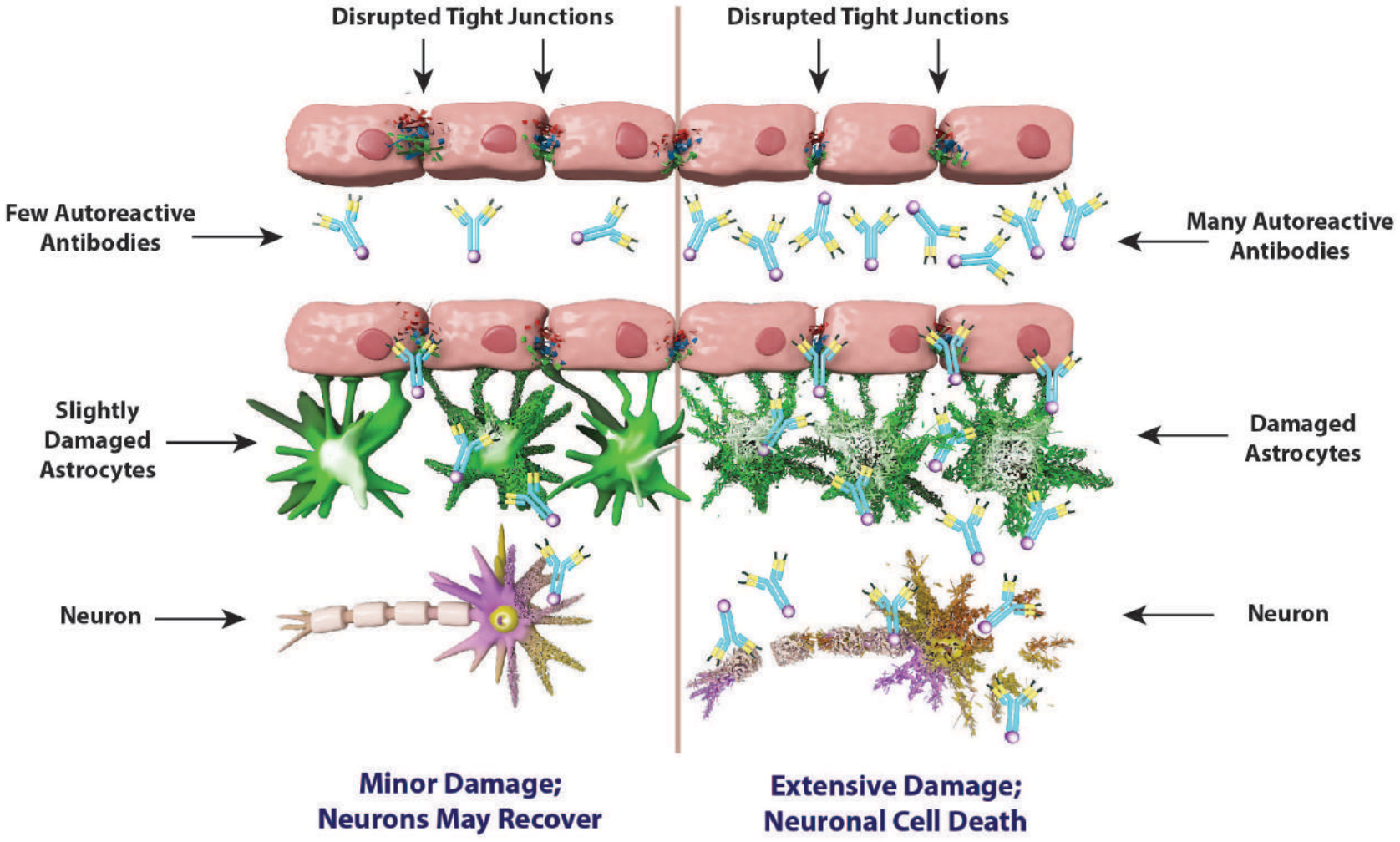
Neural Regen Res. 2015 Feb; 10(2): 203–204.

Neuroinflammation

- **Neuroinflammation** is inflammation of the nervous tissue initiated in response to a variety of cues, including infection, traumatic brain injury, toxic metabolites, or autoimmunity.
- **Circulating peripheral immune cells may pass through a compromised BBB and encounter neurons and glial cells** expressing major histocompatibility complex molecules, perpetuating the immune response.



BBB Dysfunction

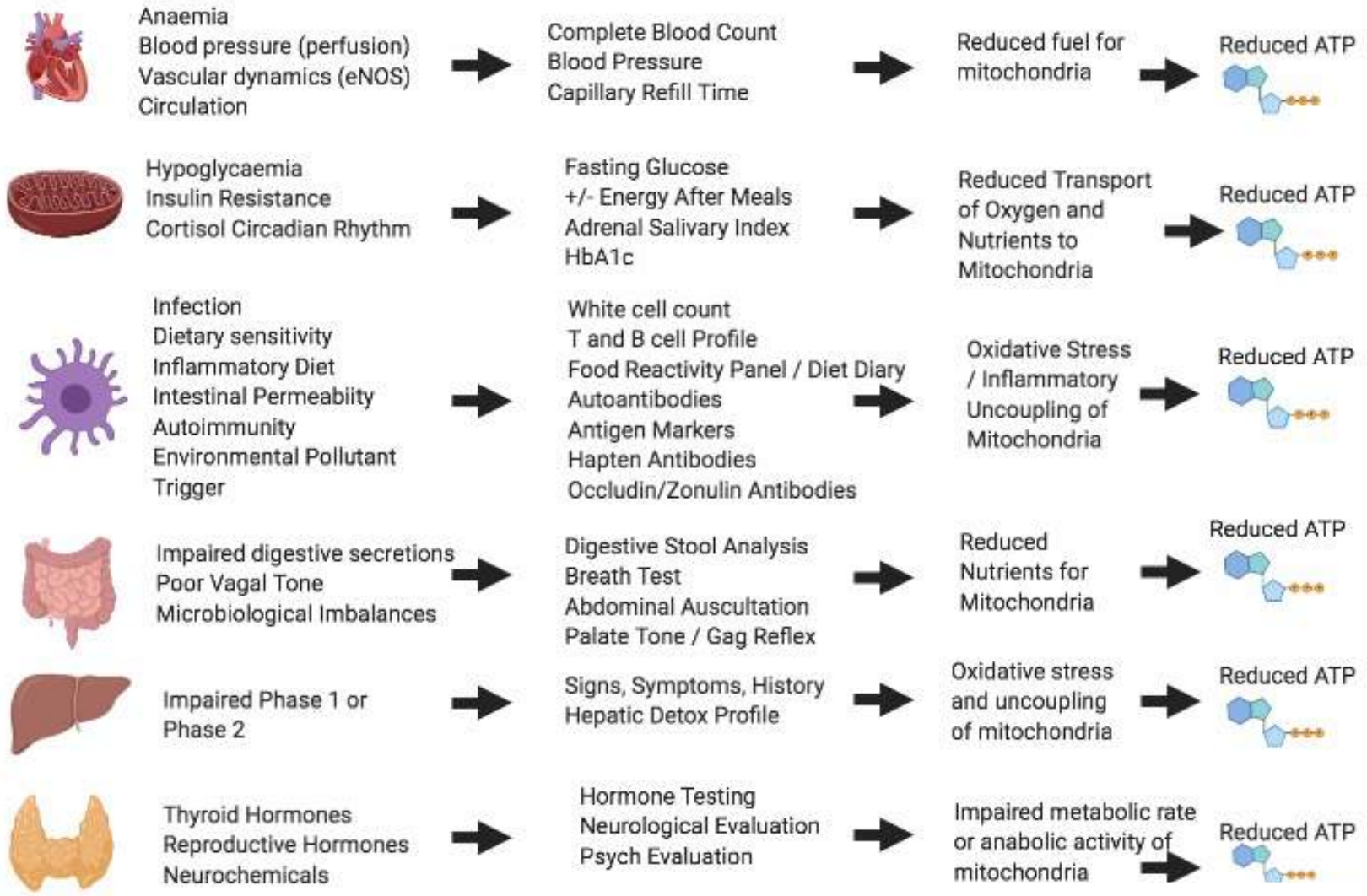


May result in neurodegeneration

Cyrex Array 20 – Blood Brain Barrier Permeability

TEST	RESULT			
Array 20 - Blood Brain Barrier Permeability Screen	**IN RANGE (Normal)**	**EQUIVOCAL***	**OUT OF RANGE**	**REFERENCE** (ELISA Index)
Blood Brain Barrier Protein IgG+IgA		1.75		0.3-2.2
Blood Brain Barrier Protein IgM	0.44			0.3-2.2

Functional Evaluation of a Fatigued Patient



Testing Considerations

- Mucosal Immune testing – Cyrex Array 14
- ASP w CAR (Genova, DUTCH)
- RBC minerals (DDI, Genova, Biolab)
- Stool / SIBO
- Blood Chemistry
- Oxidative Stress Markers
- Vitamin D
- Fat soluble vitamins (ADEK CoQ10: Genova, Biolab)
- Mould (GPL Mycotox)
- Hepatic Detox Profile (DDI)
- Glucose Management
- Sleep tracking
- Glutathione
- BBB Permeability (Array 20)

Thank You

Get in Touch

- www.robynpuglia.com – free resources, EBV e-book
- www.afmmp.co.uk – for professional mentoring
- Instagram: @robynpuglia
- Autoimmune professional mentoring program (2022) – sign up to my newsletter via my website
- Purchase the Immunity Community complete and comprehensive program to run in your own practice via my website.