



# **Biomarkers: Intestinal Permeability & Metabolic Wellness**

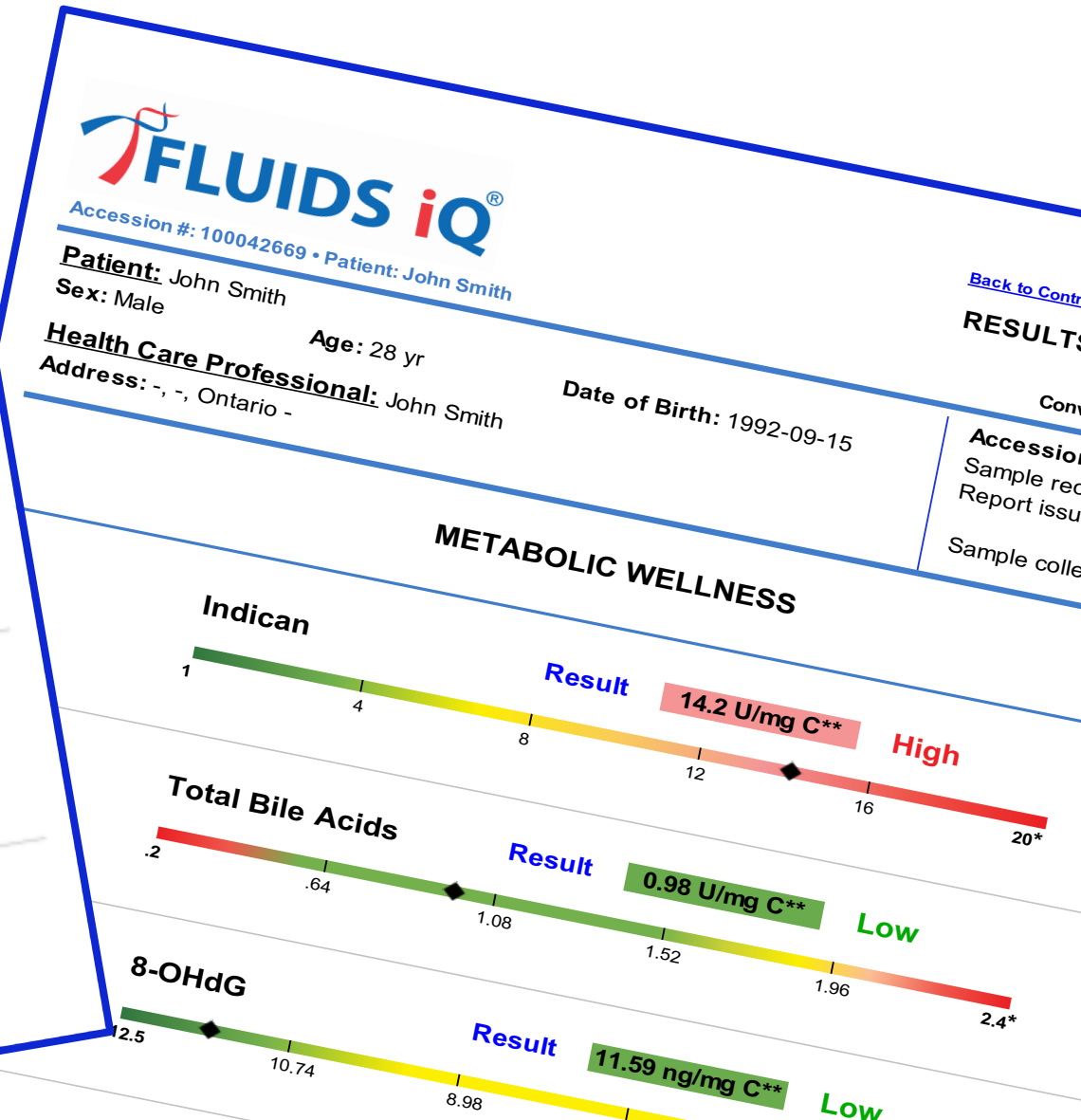
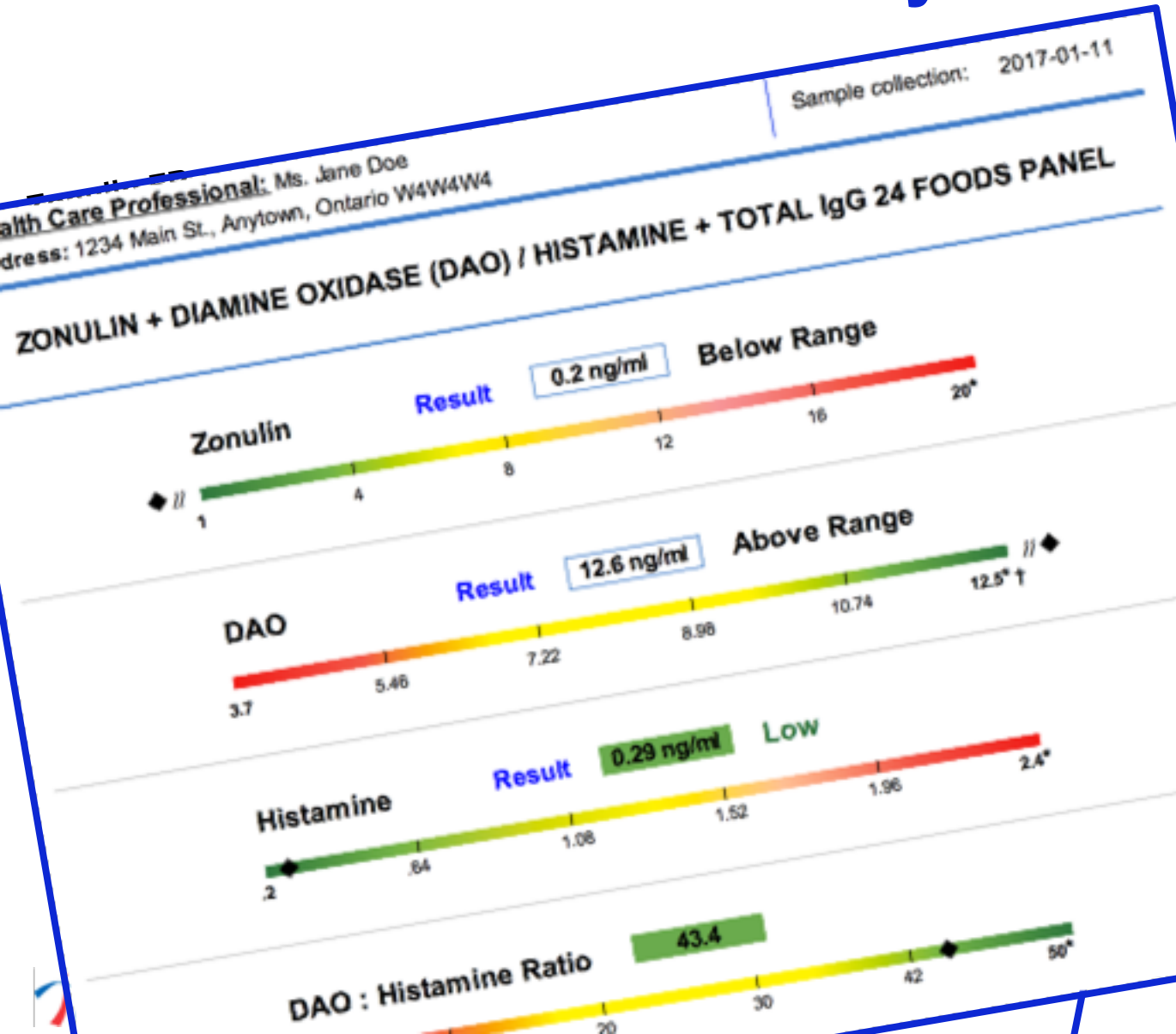
*Dr Aron Gonshor: PhD, DDS, FRCD(C), FAO*

*February 10, 2021*



2020-01-11

# Intestinal Permeability & Metabolic Wellness Reports



# INTESTINAL PERMEABILITY





# The Role of Biomarkers

1

4

**Genetic Predisposition**

**Zonulin**

Modulate or Reverse the process

**Increased Intestinal Permeability**

**Inflammatory Disease and Auto-Immunity**

**Histamine /DAO**

**Intestinal Inflammation & Immune Response**

2

**Environmental Triggers**

**Candida Suite (IgM, IgG, IgA)**

**Candida**

**Microbiome/ Dysbiosis**

3

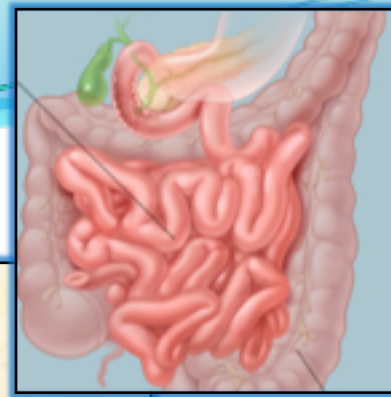
**Food Sensitivity**

Food Proteins & peptides

Infections

Toxic Chemical Products

# Intestine



- Largest surface of the body
  - ~ 30-40 M long, > 300-400 sq. M area (2 tennis courts)
- ~150 trillion bacteria - **Microbiome**
  - ~15 x the 10 trillion cells in human body
- Continuously exposed to environment
  - Bacterial/dietary antigens
- Largest number of immune cells
  - 70% of all lymphoid tissues in body
- 40% of the body's energy expenditure

*Zonulin FP*



**Increased  
Intestinal  
Permeability**

# Intestinal Absorption

- **Intestinal Mucosa: Paradoxical role**
  - **Absorb nutrients**
  - **Exclude toxins and Macromolecules**
- **Mucosal breach may lead to:**
  - **Malabsorption**
  - **Increased exposure to toxins & antigens**

# What is Intestinal Permeability ?

**Movement across the gut wall**



2 pathways

**Para(Inter)cellular Diffusion** (between cells)

**Transcellular Transport** (through the cell)



# Zonulin FP: Physiologic Role

These peptides control opening of:

Paracellular **Tight Junction** Pathway

- **Defensive mechanism: Flushes out microorganisms**
- Body's response to bacterial colonization of the small intestine

**Rapid, Reversible, Reproducible**

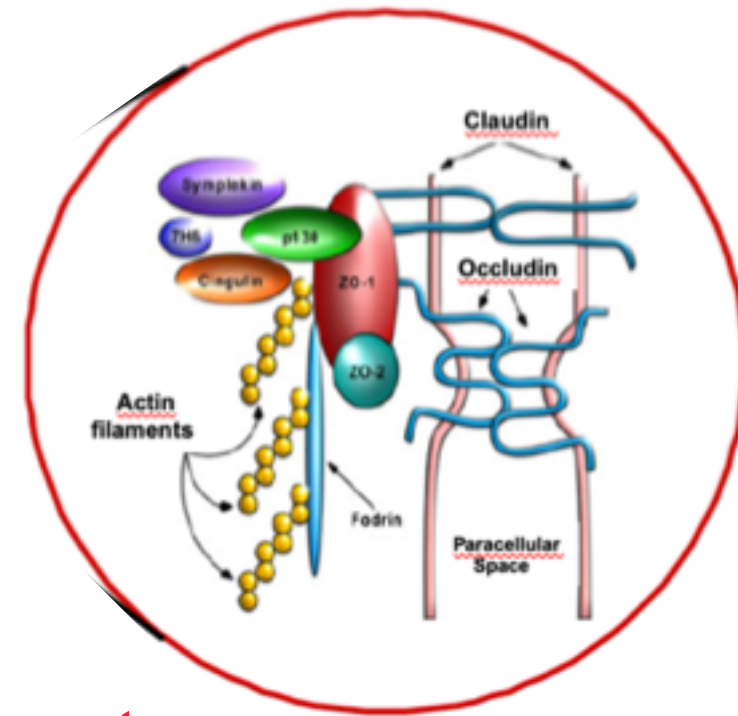
# Zonulin FP

“The only physiological modulators of intercellular tight junctions, described so far, that;

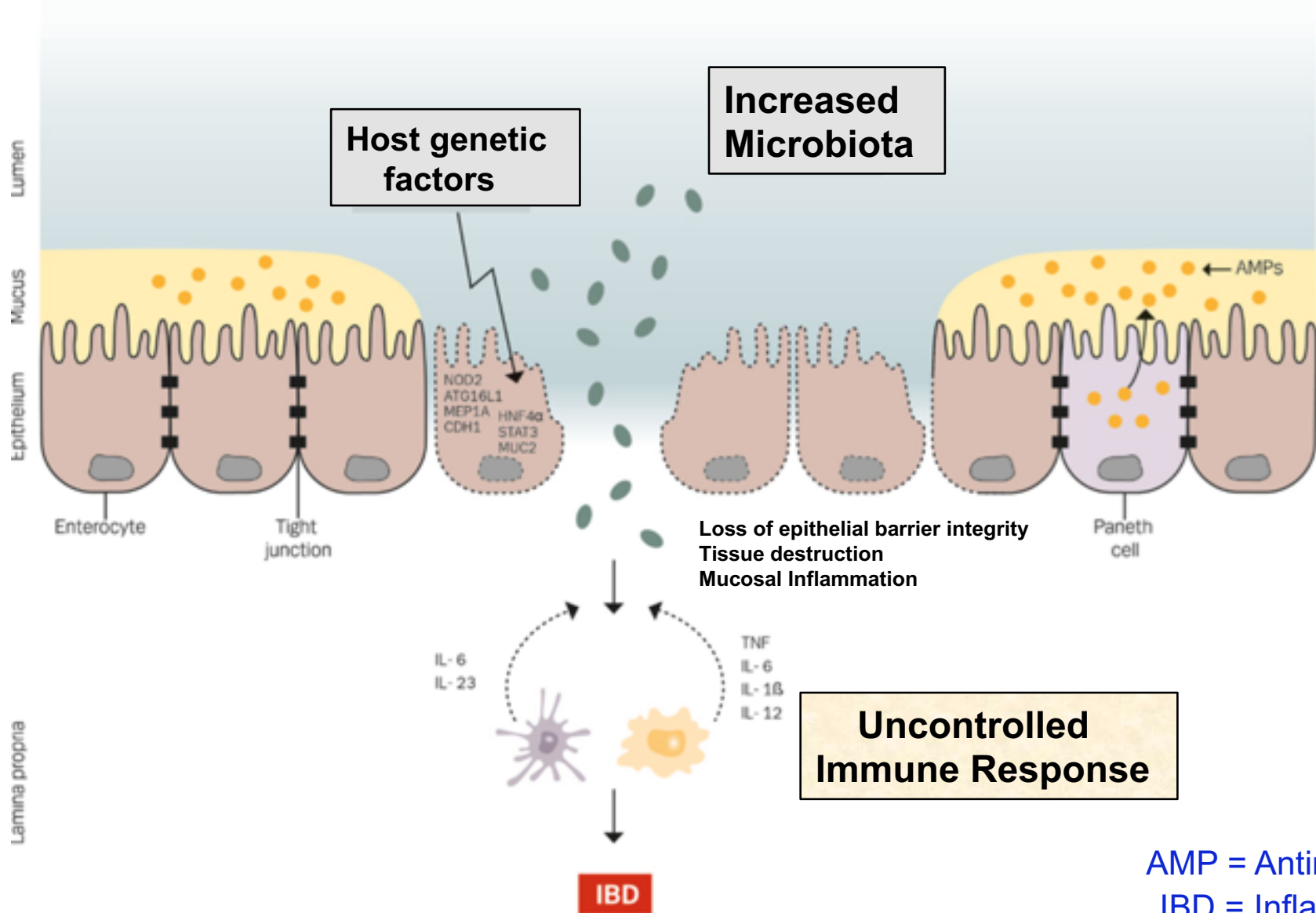
1. Helps control **macromolecule movement**
2. Modulate **tolerance/immune** response”

# Paracellular Tight Junctions (TJ)

- Once considered static. Now seen as **Dynamic**
- Adapt to developmental, physiological, pathological, and dietary circumstances
  - **Defense** against some infectious agents
  - **Absorption** of nutrients: passive
  - **Surveillance** of gut bacterial content
  - **Transport** of leukocytes (WBCs) into the gut



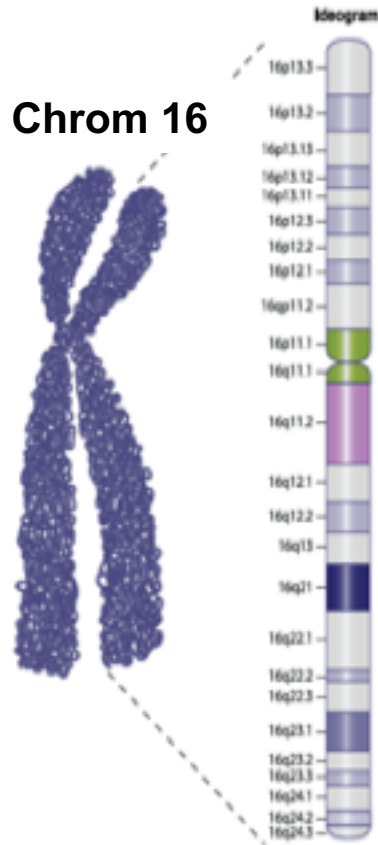
# IBD: Effect on Intestinal Permeability



# Diseases associated with Zonulin FP

- **Autoimmune Diseases**

- Inflammatory Bowel Disease
  - Chron's Disease & Ulcerative Colitis
- Celiac Disease, Diabetes (type 1)
- Rheumatoid arthritis, Lupus, Ankylosing spondylitis



- **Nervous System**

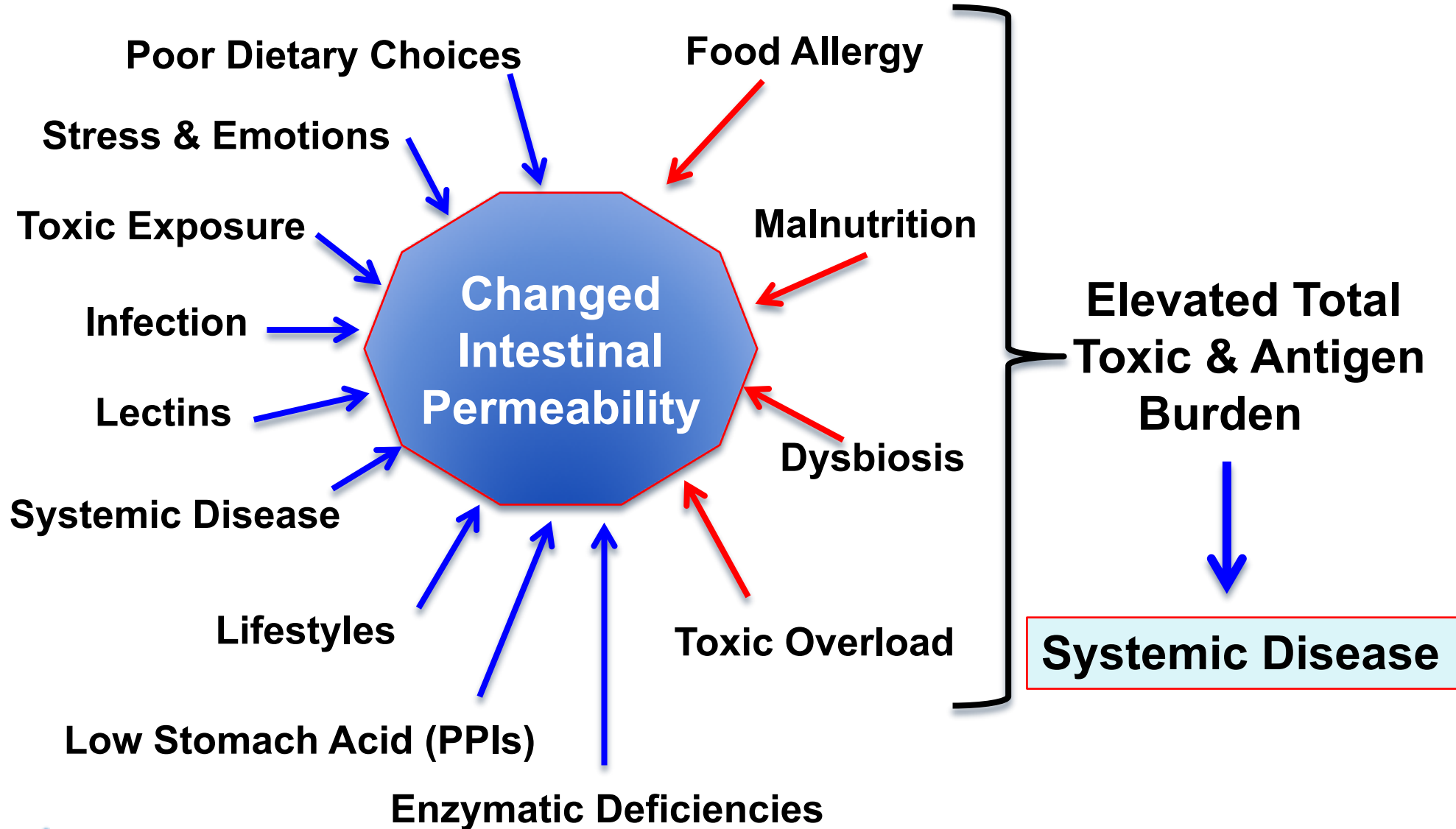
- Multiple Sclerosis
- Polyneuropathy (chronic inflam. demyelinating)
- Schizophrenia, Autism (?)

- **Cancers**

- Brain (gliomas), Breast, Ovarian,
- Pancreatic, Lung adenocarcinoma



# What affects Intestinal Permeability



# Conditions related to Intestinal Permeability

- Food Sensitivities/Allergies
- Acute pancreatitis
- Peptic Ulcers
- Cholelithiasis (gallstones)
- Diabetes (Type 1)
- Celiac disease
- Psoriasis, eczema, etc .
- Hashimoto's Thyroiditis  
(thyroid destruction)

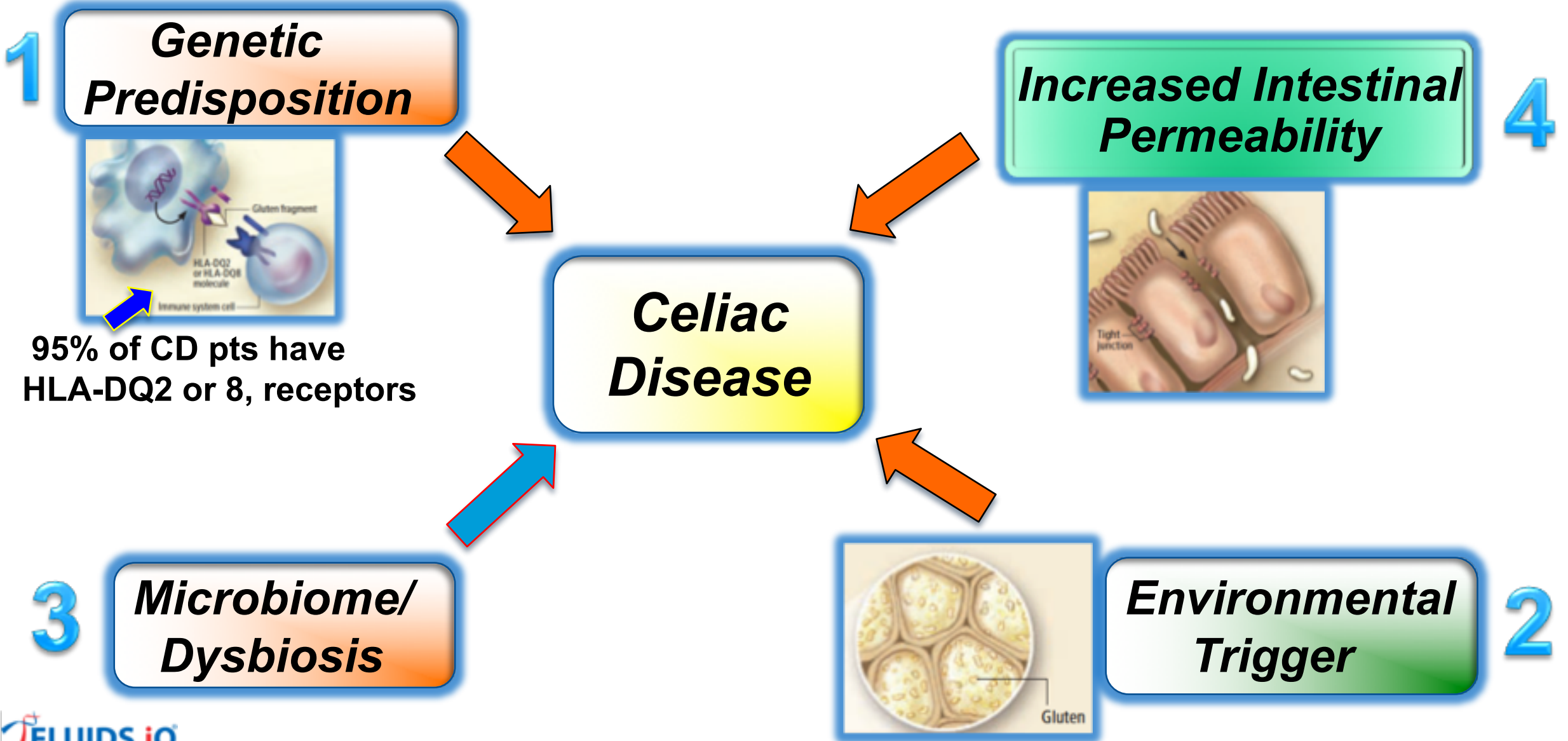
- Chronic fatigue syndrome
- IBD: Crohn's disease, UC
- SIBO
- Irritable Bowel Syndrome
- Nutritional depletion
- Chronic Alcoholism
- NSAID Therapy
- Migraines
- Protozoal Infections
  - single cell organisms

# Intestinal Permeability & Autoimmune Disease

- **Celiac Disease**
- **Inflammatory Bowel Disease (IBD)**
  - **Crohn's Disease, Ulcerative Colitis**
- **Diabetes**
- **Multiple Sclerosis**
- **Other Diseases and Conditions**

# Causes of Celiac Disease

Fasano 2009

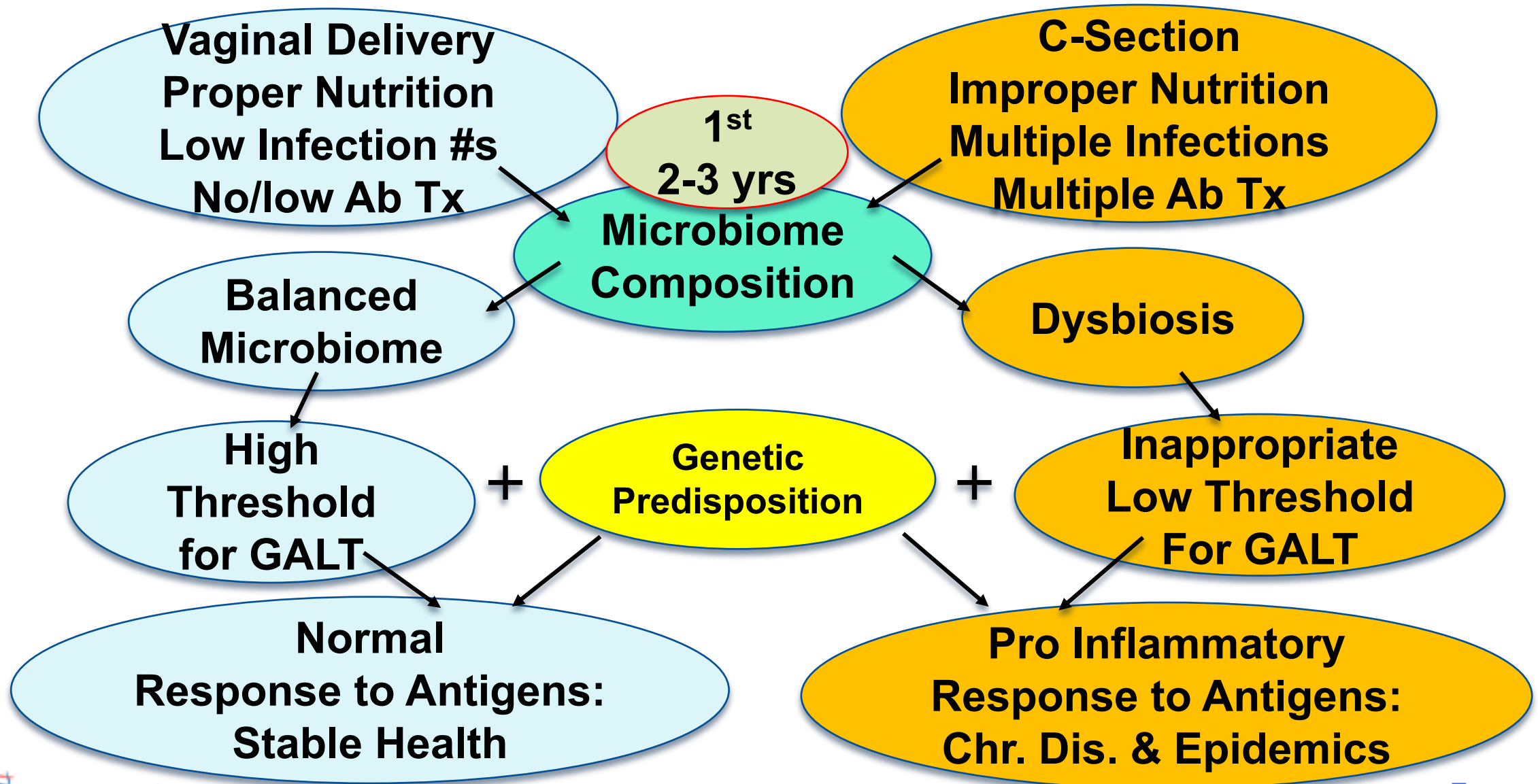


# Causes of Increased Zonulin FP & development of “Hyperpermeability”

- **Gliadin** in the diet (component of gluten)
  - Perceived as a **pathogenic organism**
- **Dysbiosis: Specifically Overgrowth**
  - **Bacterial** (SIBO), especially harmful forms
  - **Fungal** (eg; Candida) and Yeast
  - **Parasitic infections**



# Factors Driving Rise in Autoimmune Diseases



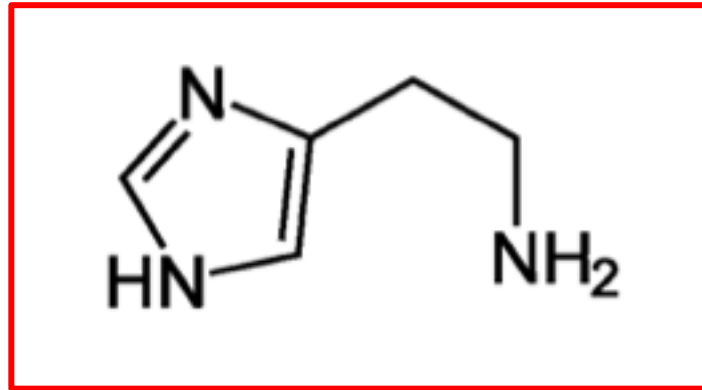
*Histamine & DAO*



**Intestinal Inflammation  
& Immune Response**

# Histamine

- **Histamine:** A Biogenic amine (2-(4-imidazolyl) ethylamine)



- Involved in the following:
  - Local immune responses
  - Regulating physiological functioning in the gut
  - Neurotransmitter

# Histamine Intestinal Metabolism

## Diamine Oxidase (DAO)

- A protein stored in cell plasma membrane vesicles. Secreted upon stimulation
- Scavenges **extracellular** histamine (e.g., after ingestion of histamine-rich food)

‘Diamine Oxidase activity is unique among intestinal mucosal enzymes, in that circulating levels can serve as **a marker of mucosal maturation and integrity**’ . Luk et al. 1980

**Decreased DAO activity indicator of intestinal mucosal damage**

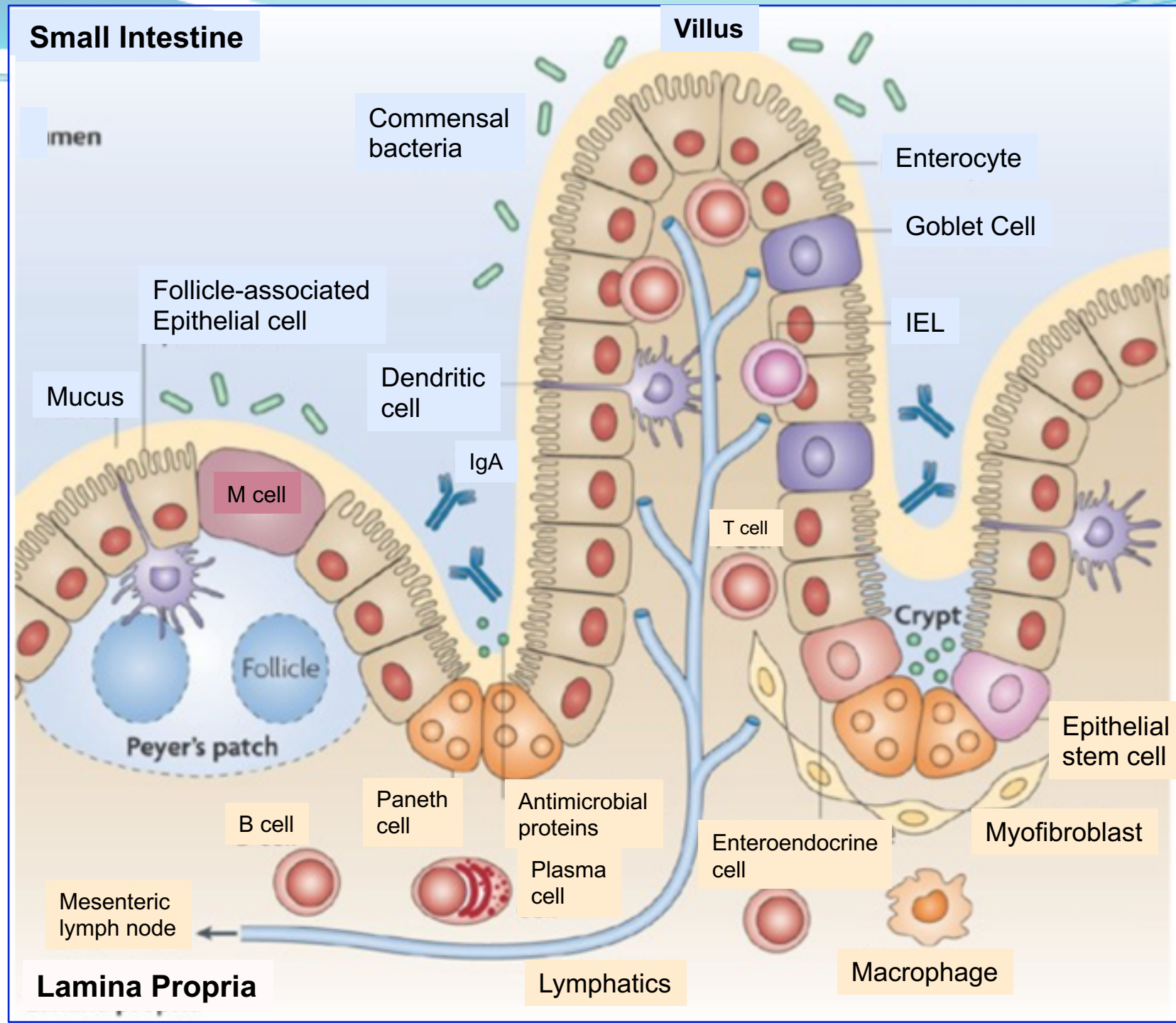
# Histamine effects & Plasma Concentration

Maintz & Novak, *Am J Clin Nutr*, '07

Histamine (ng/ml)	Clinical Effect
0-1 *	None
1-2 **	<b>Increased Gastric Acid Secretion</b> Increase in Heart Rate
3-5	Tachycardia, headache, flushing, urticaria, pruritis
6-8	Decrease in Arterial Pressure
7-12	Bronchospasm
~100	Cardiac Arrest

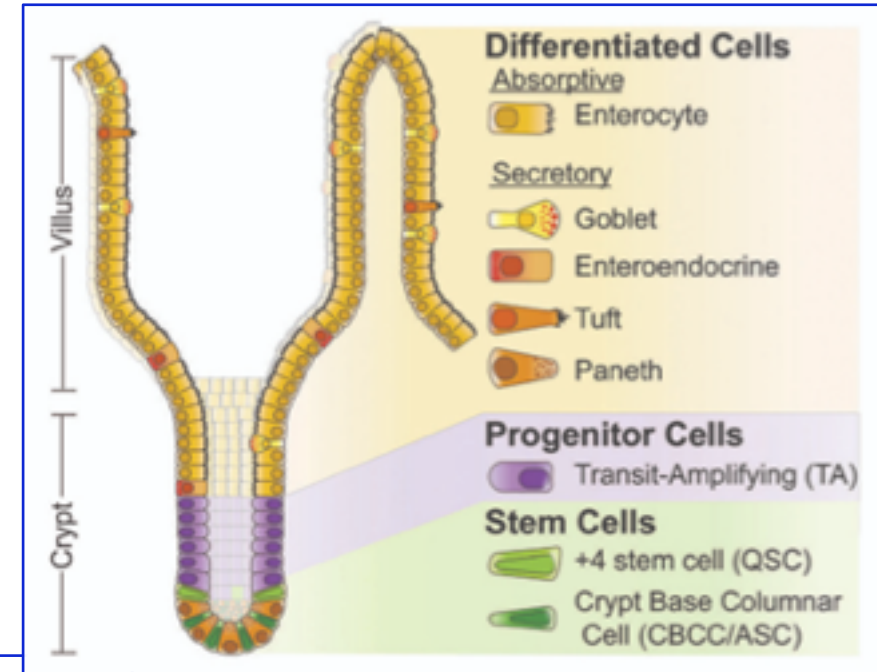
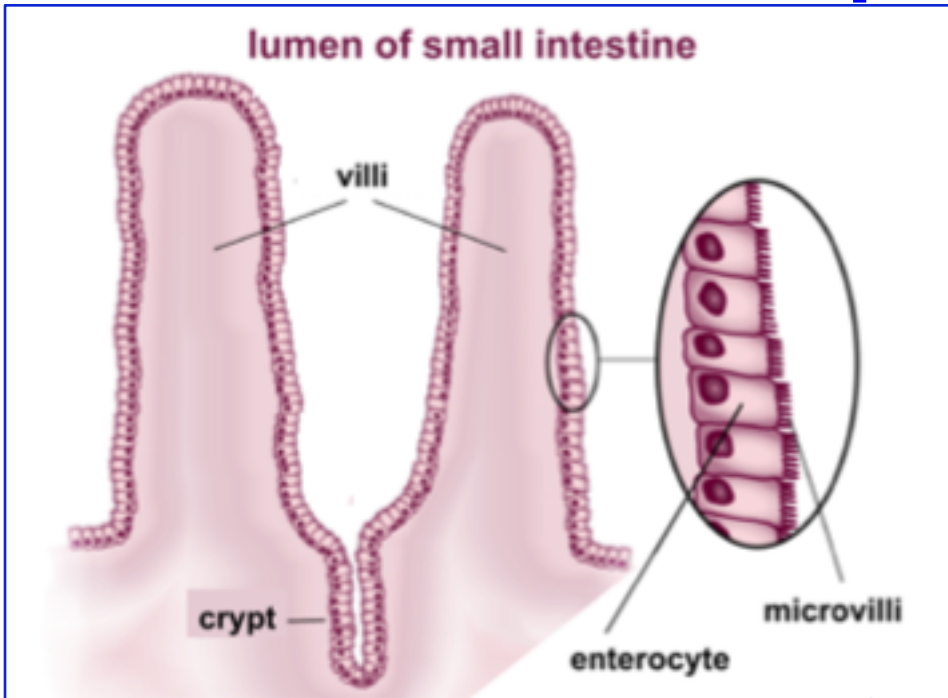


# Small Intestine: Overview

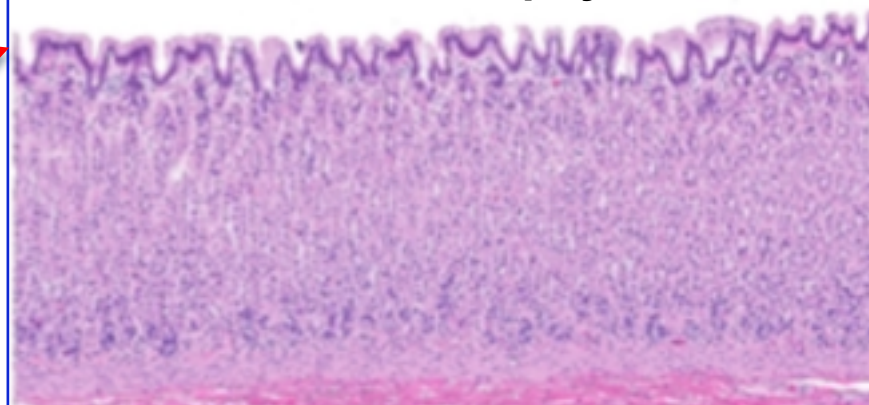


IEL = Intestinal intraepithelial T lymphocytes

# Intestinal Epithelium, Villi & Crypts

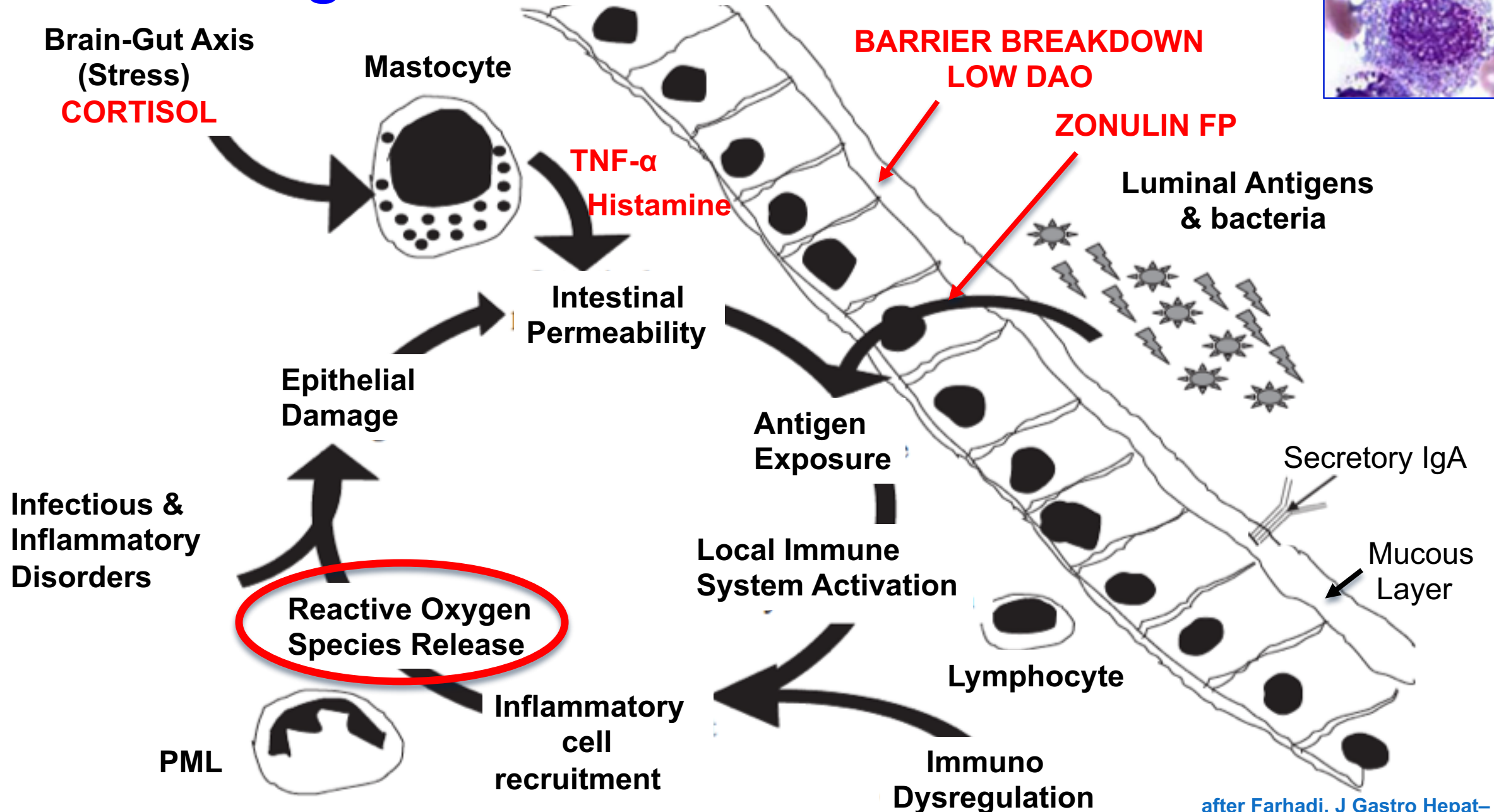
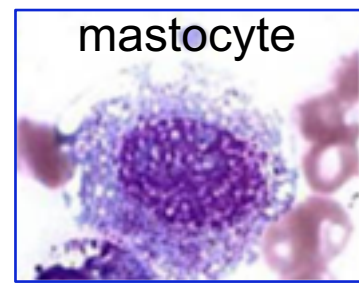


**Villous Atrophy**



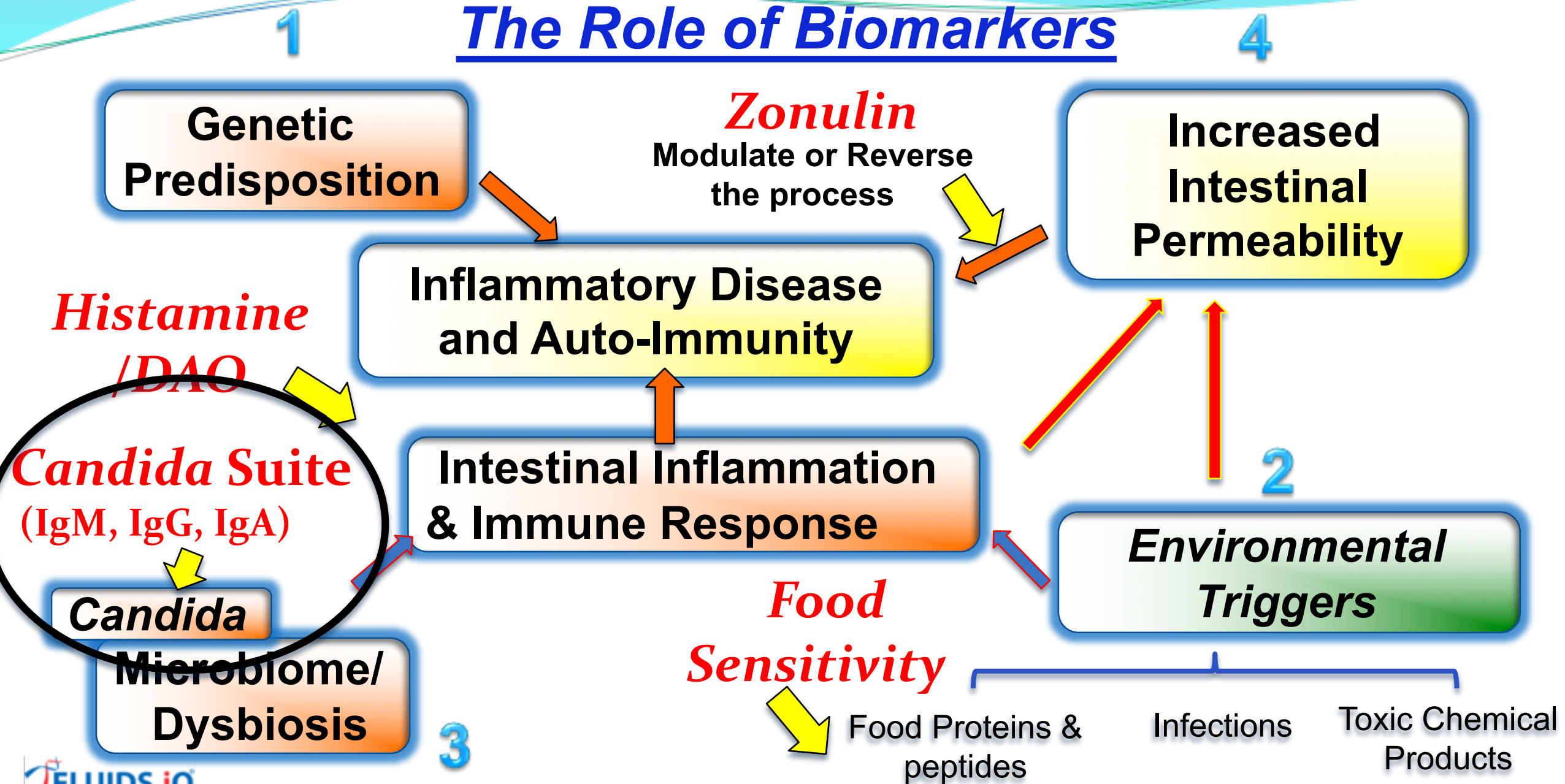
**Inflammatory Bowel Disease (IBD)**

# Pathogenesis of Intestinal Inflammation





# The Role of Biomarkers



# Candida albicans

- Yeast found in small amounts in body
  - Only fungal species that is part of 'normal' microflora
- Candidiasis: Fungal infection from genus Candida
  - Candida albicans found in over 80% of fungal isolates

Colonizes mucous membranes



Passage into host bloodstream



Humoral immune system stimulated



Antibody production against Candida albicans

# How is Candida overgrowth determined?

- **Blood Test:**

- Checks for IgG, IgA, and IgM **antibodies**. Often the **preliminary** test.
- Reflects an activated immune response to acute or chronic infections

- **Stool Test:**

- Checks for the *Candida* **antigen**, as part of a comprehensive stool analysis.
- Often significant antigen levels, since *Candida* part of commensal gut population



# Immunoglobulins (Ig<sup>s</sup>)

## IgM

- **First Ig type** produced in an infection
- **Activates Complement**

## IgG

- **Main Ig in serum, & immune response**
- **Memory response**
- Passes through vascular system & placenta
- Activates complement
- 4 Subtypes: IgG1, 2, 3, 4

## IgA

- **Primary function in mucosal tissue**
- Immune component of GI system
- Early activation of a developing food or chemical sensitivity

## IgE

- **Allergic reaction involvement**
- **Lowest concentration in serum**

# Yeast Overgrowth: *Candidiasis*

- **Candida Suite: IgM, IgG, IgA Antibodies (Abs)**

**Ab Screening Test** for Proliferation of Candida and Candidiasis

- **IgM**: Increase at the **beginning** of an infection
- **IgG**: Develop later. Indicate **past or prolonged infection**
- **IgA**: Present almost exclusively in **mucous membranes**  
When in blood, indicates **ongoing** infection

**NON REACTIVE:**  
 No significant antibody level  
 of *Candida albicans* detected.

**CANDIDA SUITE (IgM, IgG, IgA)**

Analyte	Result	Reaction	Reference Range* (U/ml)		
			Non Reactive	Indeterminant	Reactive
Candida IgM	10.26	Indeterminant	< 9	9 - 11	> 11
Candida IgG	7.25	Non Reactive	< 9	9 - 11	> 11
Candida IgA	14.25	Reactive	< 9	9 - 11	> 11

\*Reference range derived from a normal distribution of results, encompassing 95% of a randomly selected population

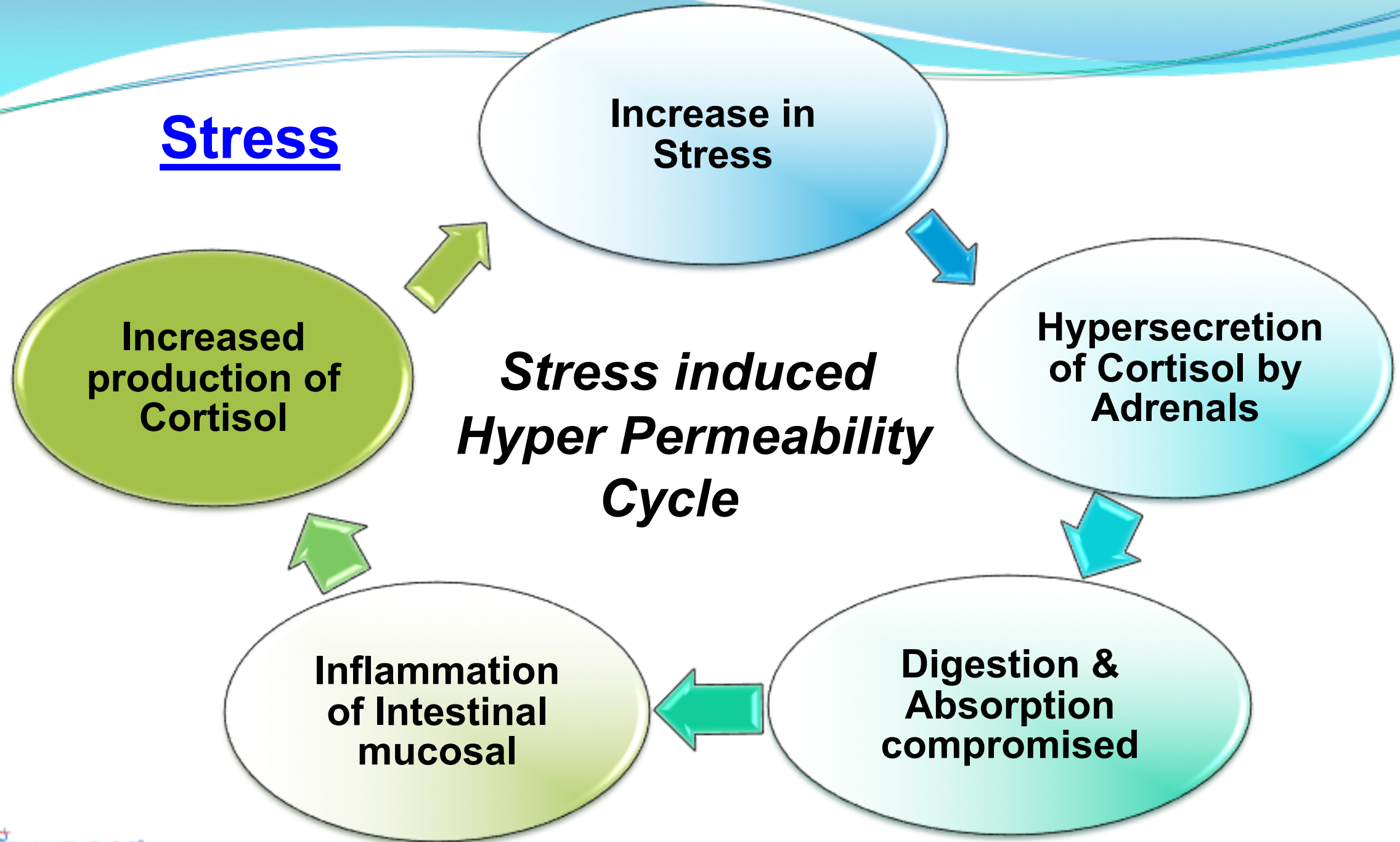
**REACTIVE:**  
*Candida albicans* detected.  
 May indicate past, active or prolonged  
 infection, depending on antibody level.

**INDETERMINANT:**  
 Presence of antibody remains  
 uncertain or indefinite, due to cross  
 reactivity with other *Candida* species  
 that may have pathological potential.

**Non Reactive:** Values are considered Non Reactive when no significant level of the *Candida albicans* antibody has been detected.



# Stress



# Complementary Assessment

Usefulness  
Rating  
\*\*\*\*\*

## 1. Permeability Changes: Damage to Tight Junctions

Primary Screening

*Blood Zonulin FP*

Usefulness  
Rating  
\*\*\*\*\*

## 2. Inflammation & Permeability Changes

- **Histamine**: Shows if there is inflammation
- **Diamine Oxidase (DAO)**:
  - Is Histamine being broken down?
  - If DAO low:
    - **Permeability** changes?
    - If histamine high: Diet/Supplements

# Complementary Assessment (Cont<sup>d</sup>)

Usefulness  
Rating  
\*\*\*\*\*

## 3. Activation of Immune Response

- **Immunoglobulin G (Total IgG ) Food Sensitivity**
  - Identifies Ab/antigen complexes causing inflammation
- **Candida Albicans Suite (IgG, IgA, IgM)**
  - Overgrowth a common manifestation of dysbiosis

Usefulness  
Rating  
\*\*\*\*\*

## 4. Stress & Permeability/Absorption

- **Salivary Hormonal profiles**
  - E2, Pg, T, DHEA-S, Diurnal C, and C/DH ratio
  - Adrenal fatigue and Stress index



# **METABOLIC WELLNESS**

**DIGESTION, LIVER DETOXIFICATION, OXIDATIVE STRESS**



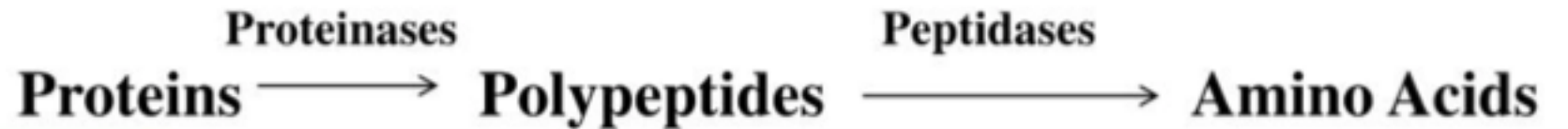
# METABOLIC WELLNESS

**DIGESTION,** LIVER DETOXIFICATION, OXIDATIVE STRESS

# Degradation of N-Compounds

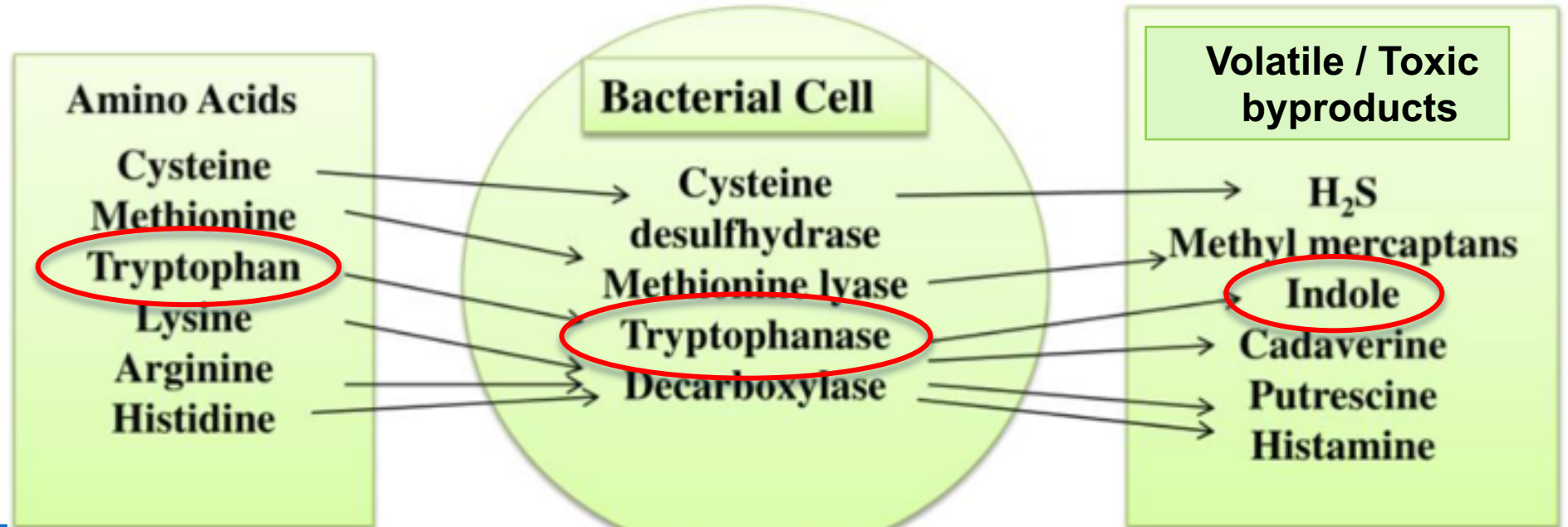
Normal Protein Metabolism

Proteolysis

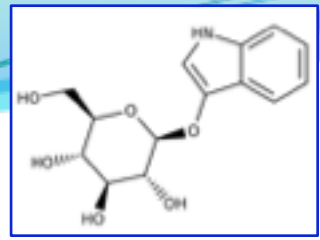


Abnormal Protein Metabolism

Putrefaction



# Indican



Tool to monitor protein digestive efficiency.

- Normal Function:
  - **Small quantities of Indole** formed by bacterial metabolism of AA **Tryptophan**
  - Most excreted in feces. Some absorbed. Processed to indican in liver.
  - Excreted in urine. Trace amounts of indican found in urine samples
- **Gut Dysfunction: Dysbiosis**
  - Anaerobic intestinal bacterial decomposition of proteins (putrefaction)
  - **High amounts of 'Indole' by-product created**
  - Absorbed into blood stream. Processed to indican in liver
  - Increased levels of urinary indican

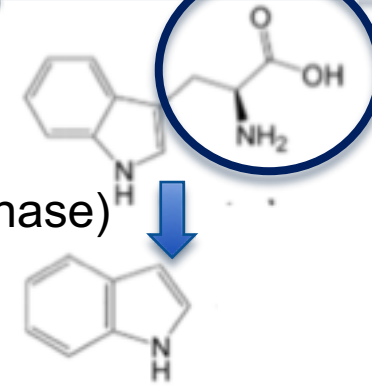
# Indican

Intestine

Partially digested food:  
including **Tryptophan**

Anaerobic Bacteria (Tryptophanase)

**Indole**



Portal Circulation

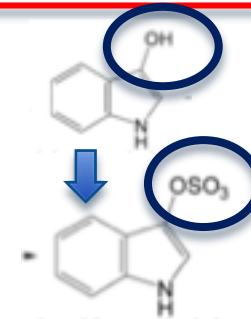
Liver

**Indoxyl** (3-hydroxyindole)

Conjugated with Sulfuric Acid

**Indoxyl Sulfate** (3-sulfonoxyindole)

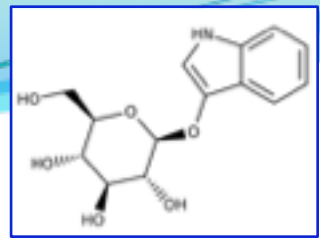
**Indoxyl Potassium Sulfate = Indican**



Kidney

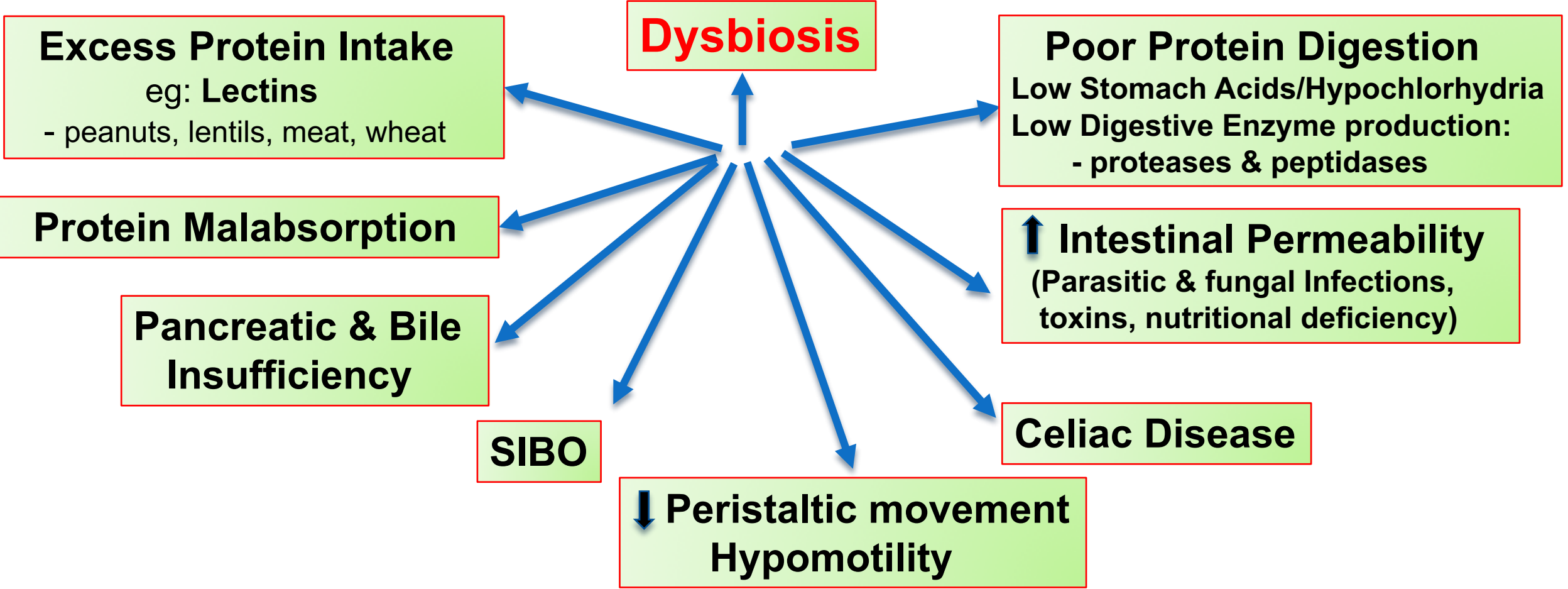
Urine

**Indican**



# Indican

Conditions creating elevated urinary indican





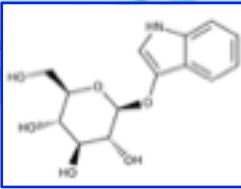
# Conditions with Elevated Urinary Indican Levels

- Inflammatory bowel disease
- Celiac disease
- Hypochlorhydria, Achlorhydria
- Gastric ulcer
- Biliary & intestinal obstruction
- Jejunal diverticulosis

# If Urinary Indican Results are Elevated?

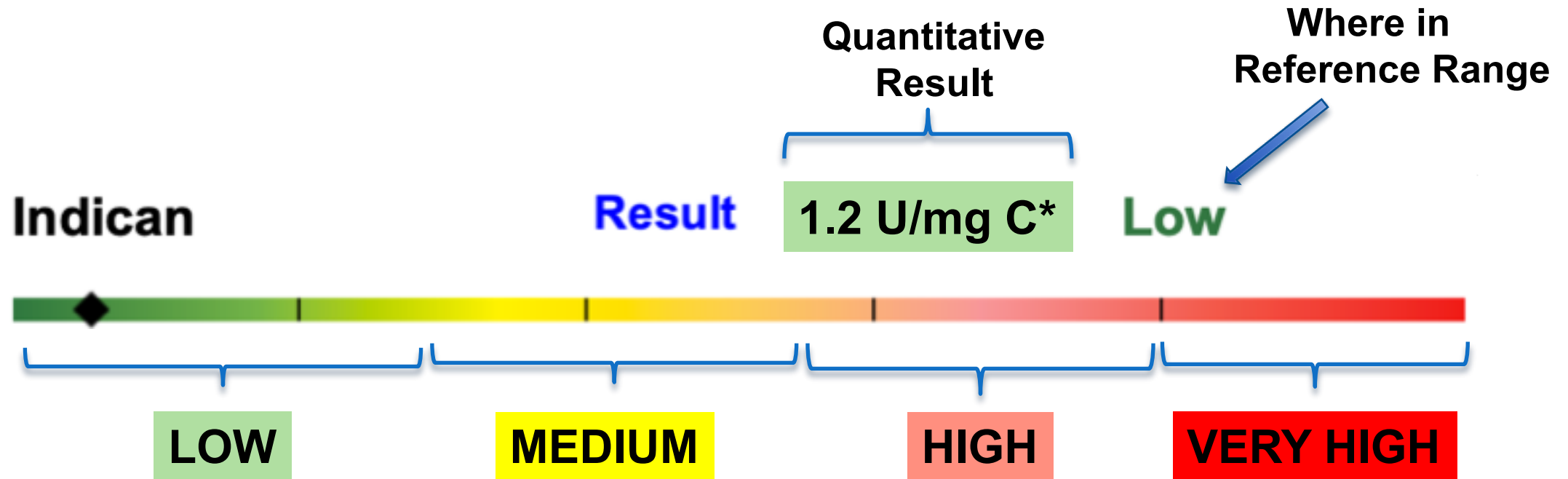
- Starting point for further investigation into gut health
- Possible Directions:
  - Optimizing levels of stomach acids
  - Replenishment of digestive enzymes
  - Probiotic supplementation or other gut support
  - Further testing:
    - SIBO
    - Comprehensive Digestive Stool Analysis (CDSA)





# Urinary Indican

## FLUIDS iQ Report

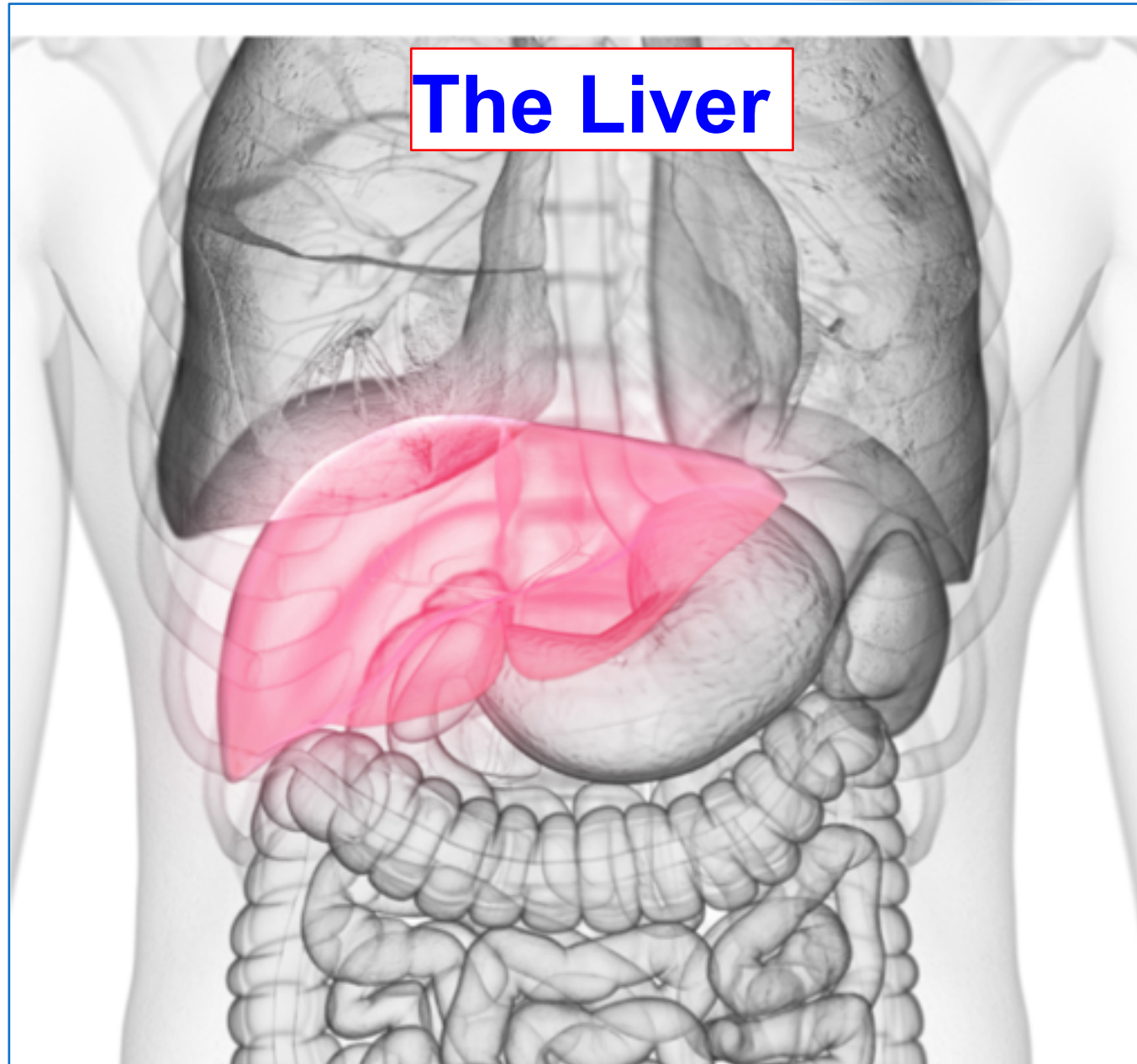


\*U/mg C = U/mg Creatinine

# METABOLIC WELLNESS

DIGESTION, **LIVER DETOXIFICATION**, OXIDATIVE STRESS

# The Liver



# Liver Functions

## Metabolism

- Synthesis: AAs, Proteins & Cholesterol
- Production of bile (from cholesterol)
- Breakdown alcohols & medications
- Regulation of hormones

## Storage/Regulation

- Fats, proteins,
- Glycogen - glucose
- Vitamins (absorption)
- Copper, iron



Over 500 functions  
& metabolic  
processes

## Immunity

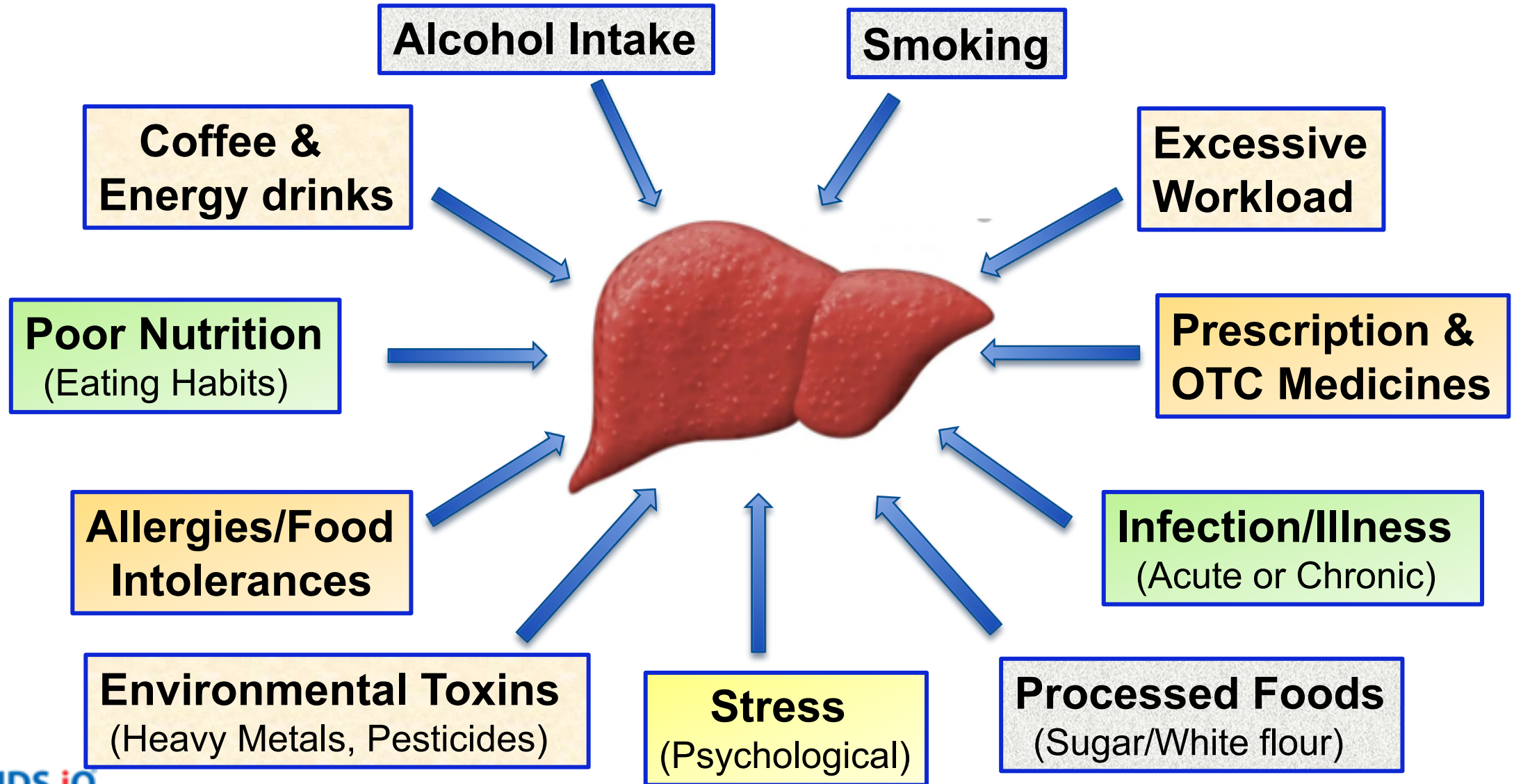
- Destroys bacterial pathogens from gut

## Blood

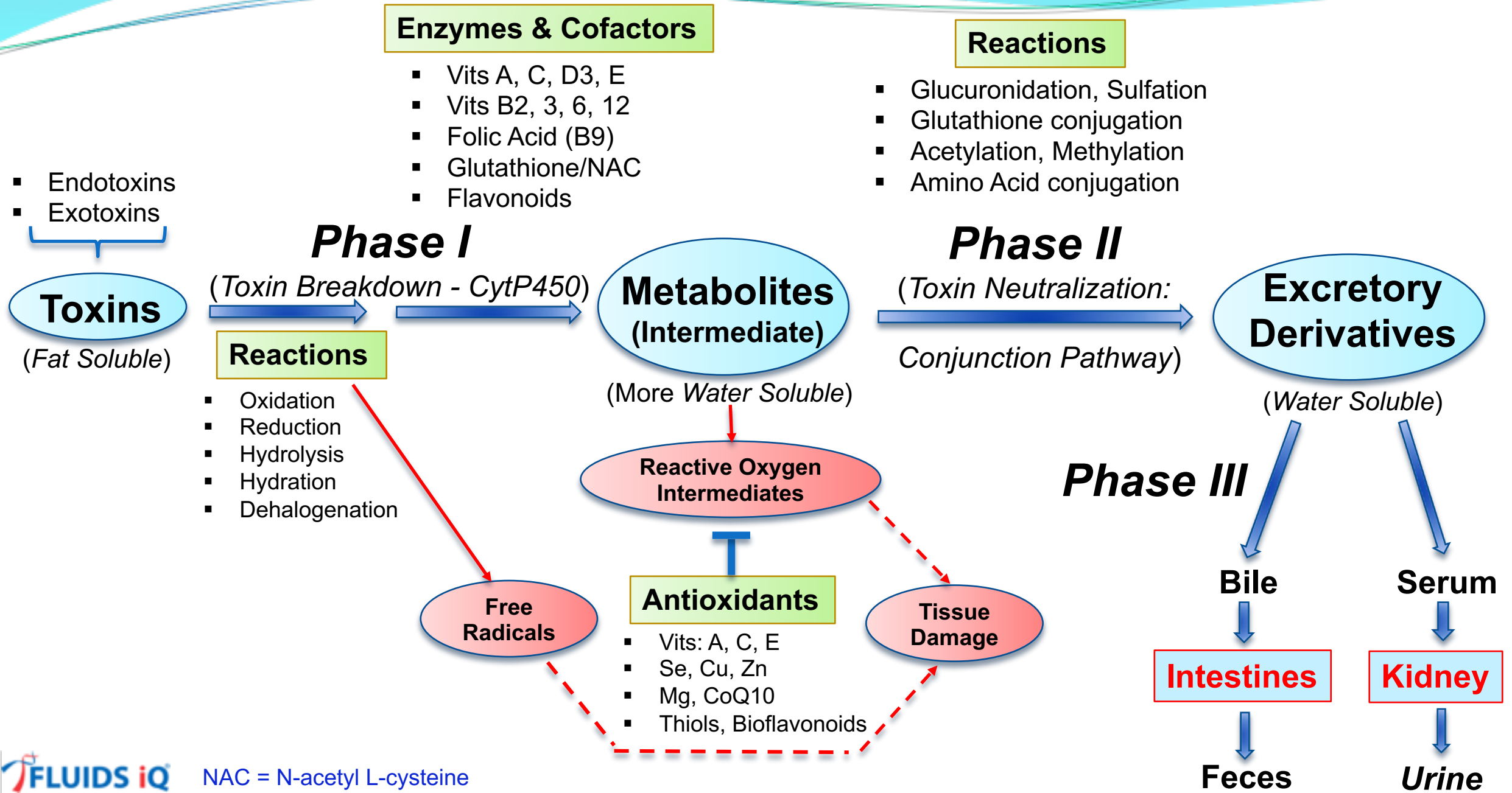
- Deactivation of toxins
- Creation of clotting factors
- Decomposition old/damaged RBCs
- Regulation **glucose**/fats/AAs



# Daily Life Overloads: Liver Dysfunction



# Liver Detoxification: Pathways



# Bile Acids (BAs)

- 12 types: Primary types – **Cholic Acid & Chenodeoxycholic Acid**
- BA levels in feces, blood, urine, & bile: markers for various diseases such as hyperlipidemia, cholestasis, gall stones, colon cancer, others.
- BAs also exist in a sulfate salt form - bile acid sulfates
  - BA sulfation increases their solubility & decreases intestinal absorption, thereby enhancing fecal and urinary excretion.



# Bile Acids

Abbreviation	Bile Acid Name	Bile Acid Type
CA	Cholic acid	Primary
CDCA	Chenodeoxycholic acid	Primary
DCA	Deoxycholic acid	Secondary
GCA	Glycocholic acid	Glyco-conjugated
GCDCA	Glycochenodeoxycholic acid	Glyco-conjugated
GDCA	Glycodeoxycholic acid	Glyco-conjugated
GLCA	Glycolithocholic acid	Glyco-conjugated
GLCAS	Glycolithocholic acid sulfate	Glyco-conjugated
GUDCA	Glycoursodeoxycholic acid	Glyco-conjugated
HDCA	Hyodeoxycholic acid	Secondary
LCA	Lithocholic acid	Secondary
MCA (a)	$\alpha$ -Muricholic acid	Primary (mouse)
MCA (b)	Muricholic acid, beta	Primary (mouse)
MCA (o)	Muricholic acid, omega	Primary (mouse)
TCA	Taurocholic acid	Tauro-conjugated
TCDC	Taurochenodeoxycholic acid	Tauro-conjugated
TDCA	Taurodeoxycholic acid	Tauro-conjugated
TLCA	Taurolithocholic acid	Tauro-conjugated
TLCAS	Taurolithocholic acid sulfate	Tauro-conjugated
TMCA (a+b)	Tauromuricholic acid (alpha+beta)	Tauro-conjugated (mouse)
TUDCA	Tauroursodeoxycholic acid	Tauro-conjugated
UDCA	Ursodeoxycholic acid	Secondary

# Total Bile Acid (TBA) Colorimetric Assay

- Bile Acids (BAs): 12 types
  - Two primary BA types: cholic & chenodeoxycholic acids
  - Dehydroxylated into two 2<sup>nd</sup>ary BAs
  - These 4 are conjugated to taurine or glycine: 8 different conjugated BAs
- TBA is a marker for screening & prognosis of:
  - Hepatobiliary disease
  - Liver parenchymal damage and cholestasis (reduced or blocked flow of bile)
- Increase of TBA indicates the risk of:
  - Viral hepatitis, cirrhosis, alcoholic liver disease,
  - Drug-induced liver injury, cholestasis

# Detection Principle

## Recycling Enzyme Immunoassay

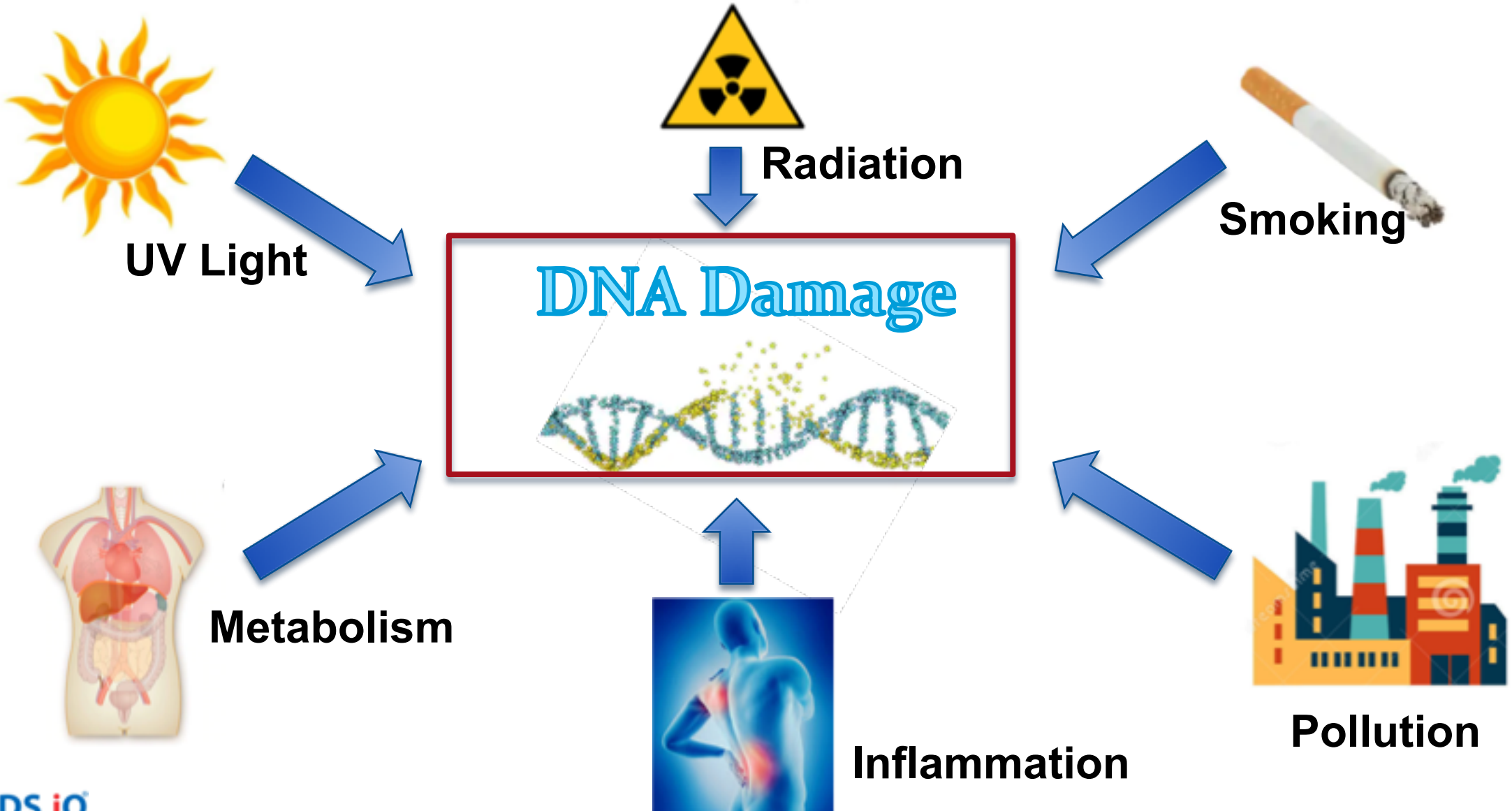


- Optical Density (OD) measured at 340 nm
- Absorbance changes linearly proportional to BA concentration
- **Result: Quantitative measure of TBA concentration in urine**

# METABOLIC WELLNESS

DIGESTION, LIVER DETOXIFICATION, **OXIDATIVE STRESS**

# Causes of Cell & DNA Damage





***A Major Cause of  
Cell & DNA Damage***



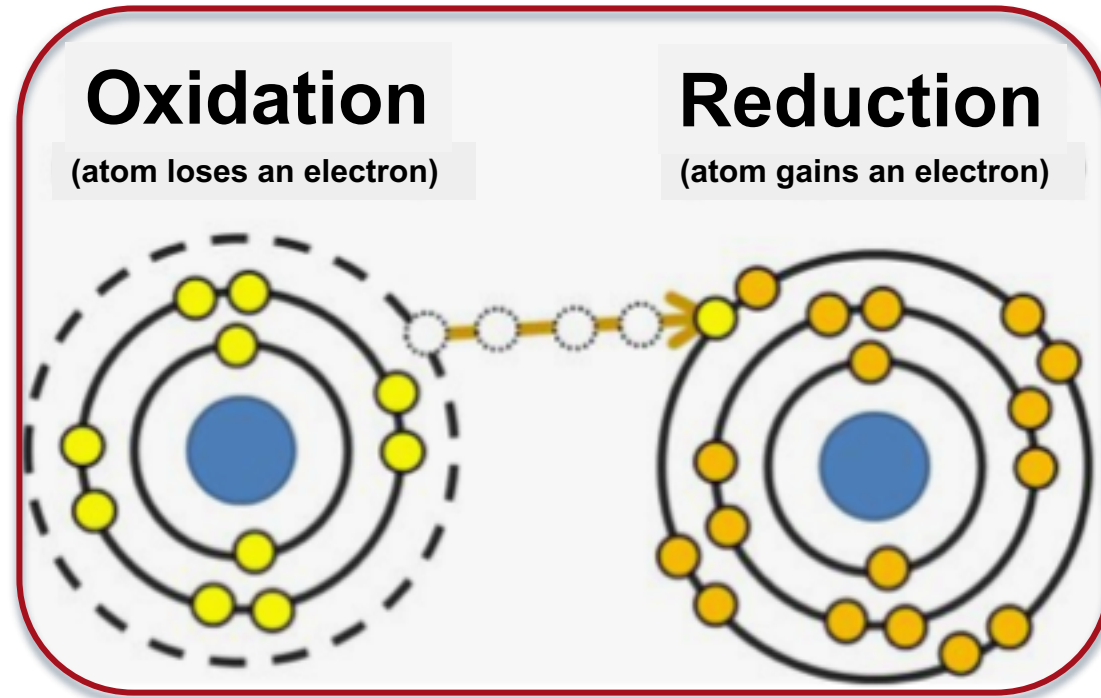
***Oxidative Stress:***

***What is it?***



# Free Radicals

- Any species that contains one or more unpaired electrons & participates in biochemical reactions
- Removing electrons: “**Oxidation**”
- Receiving electrons: “**Reduction**”
- Electron transfer: “**Redox Reaction**”



# Oxidative Stress (OS)

Marciano et al - 2017

- **Free radicals** produced in cells during normal metabolic processes. **Antioxidants** neutralize these free radicals

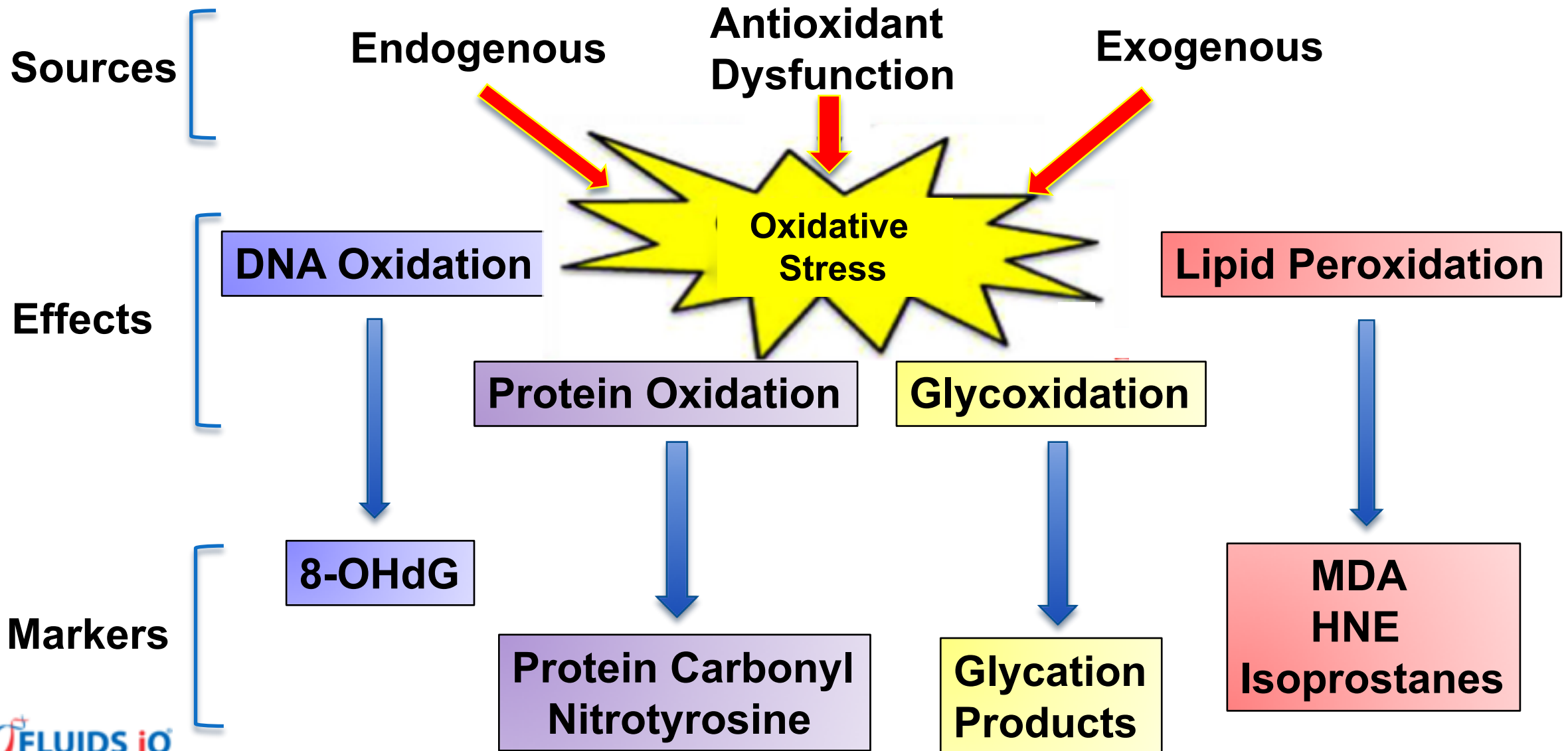


- Imbalance between **Reactive Oxygen / Nitrogen Species** (ROS / NOS), & system's ability to **Detoxify** the reactive intermediates or **Repair** the damage



***Oxidative Stress***

# Oxidative Stress - Cellular Damage - Markers



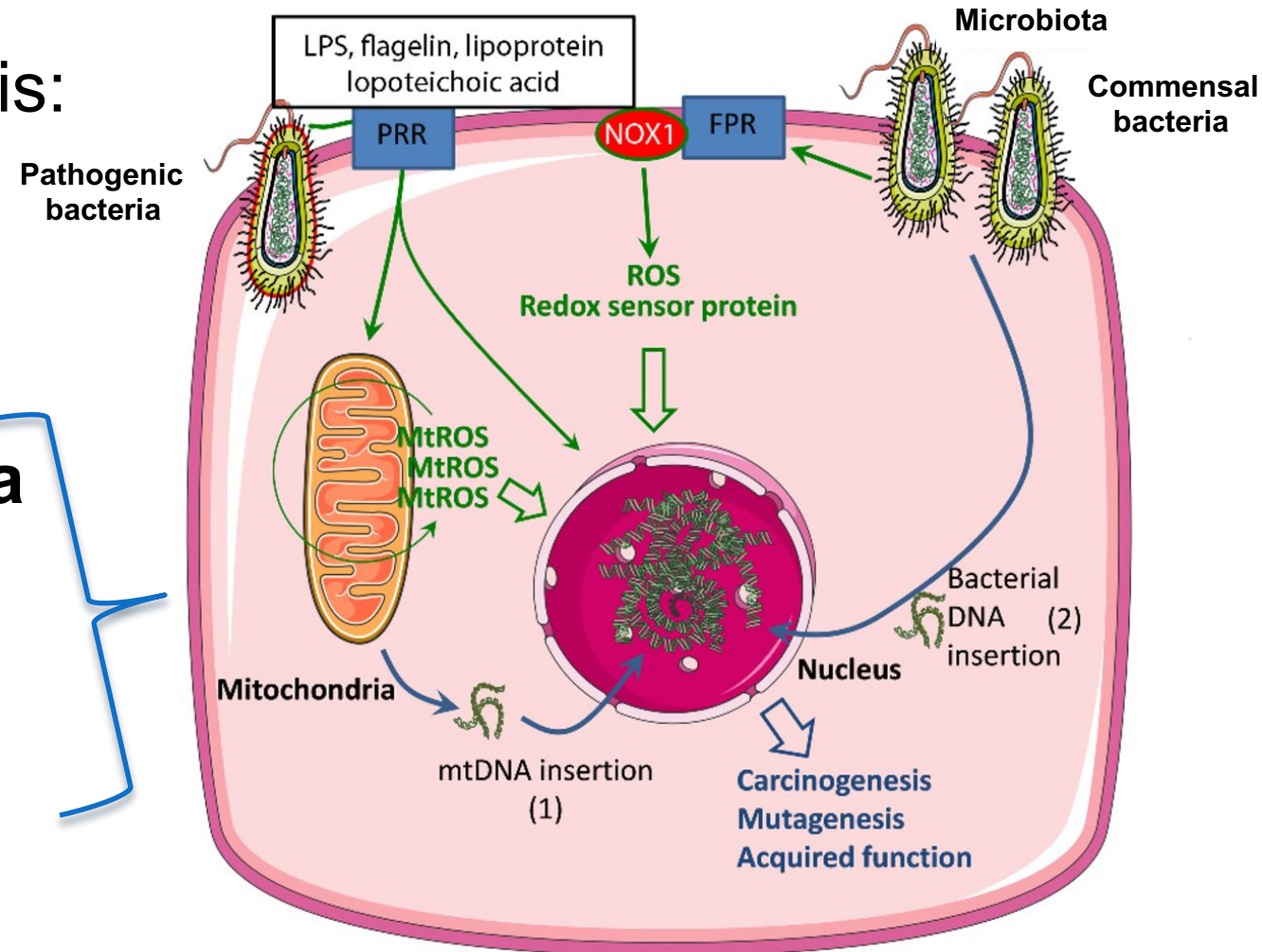
# 1. DNA Oxidation

St Georges-Chaumet - 2016

- ROS: Creation of DNA mutagenesis:  
Induce strand breaks, purine oxidation, & protein/DNA X-linking

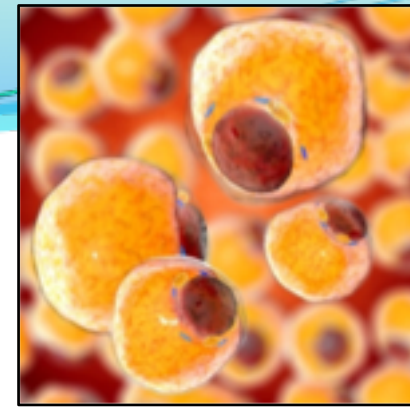
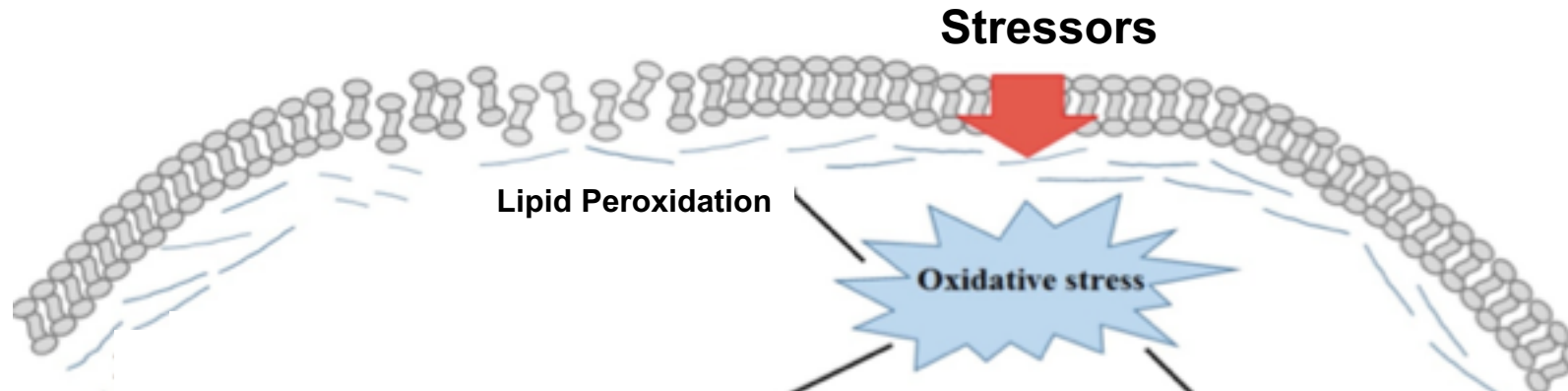
- Commensal & pathogenic **bacteria** target host cell through ROS regulation and DNA insertion.

**Mitochondrial (1)**      **Bacterial (2)**





## 2. Lipid Peroxidation



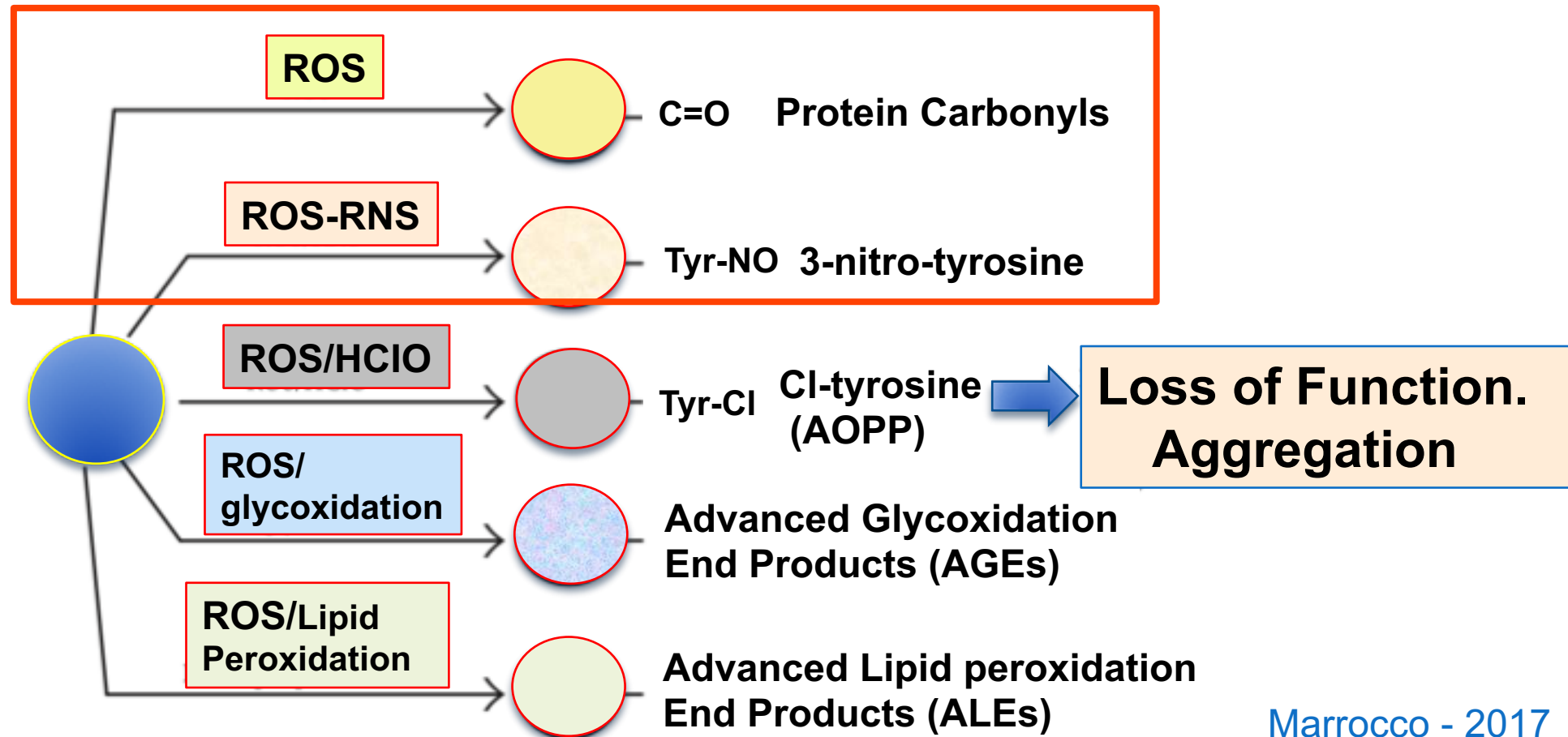
P Chagochkin

- ROS oxidize PUFAs in cell membranes:
- **Lipid Peroxidation** chain reaction starts:
  - Reduction in maintenance of concentration gradient
  - Increased **membrane permeability**
  - Inflammation
  - Creation of other free radicals & **DNA Oxidation damage**

# 3. Protein Oxidation

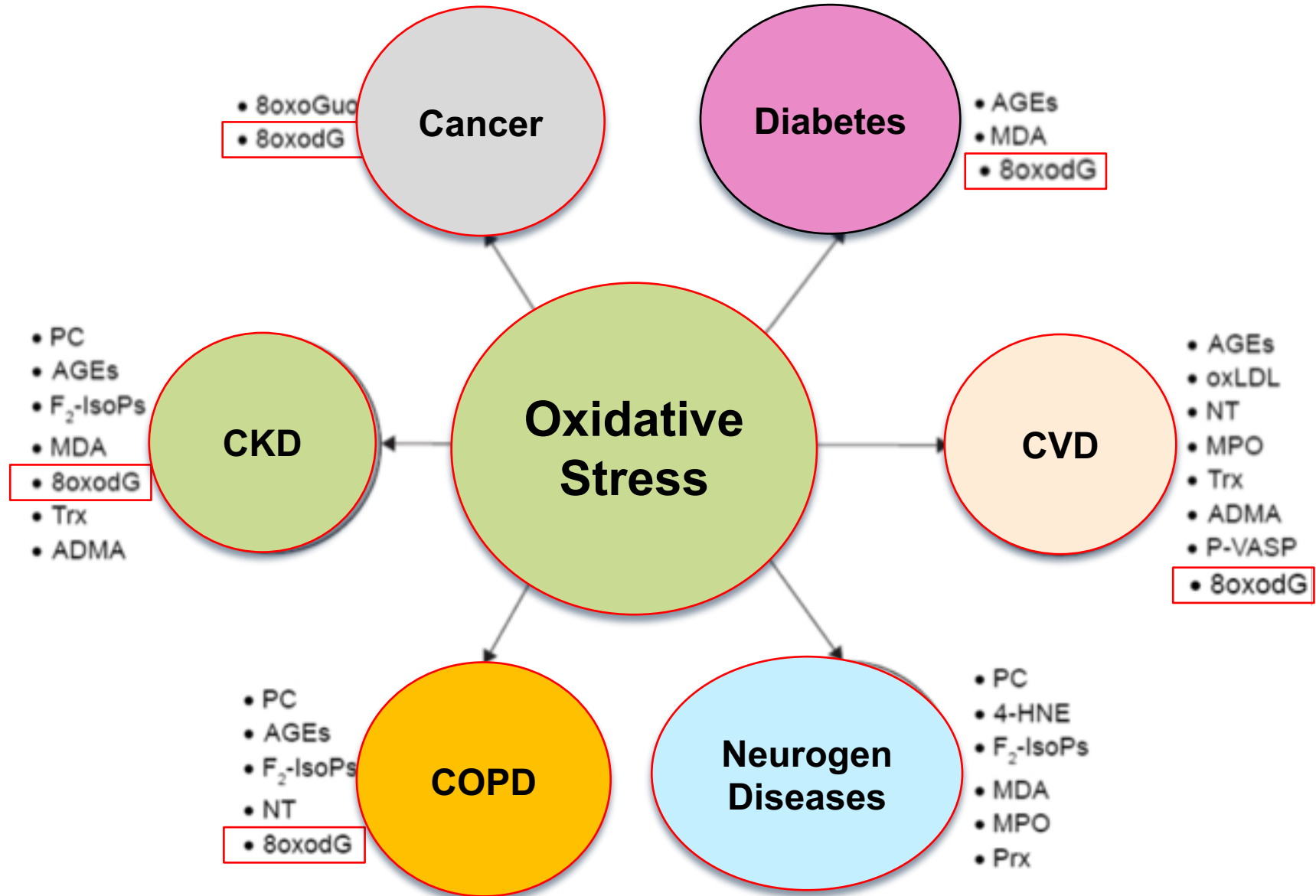
- ROS oxidize proteins and inhibit the proteolytic system.
- Severely oxidized proteins are **not degraded and accumulate in cells**

Irreversible  
Oxidative  
Modification  
of Proteins

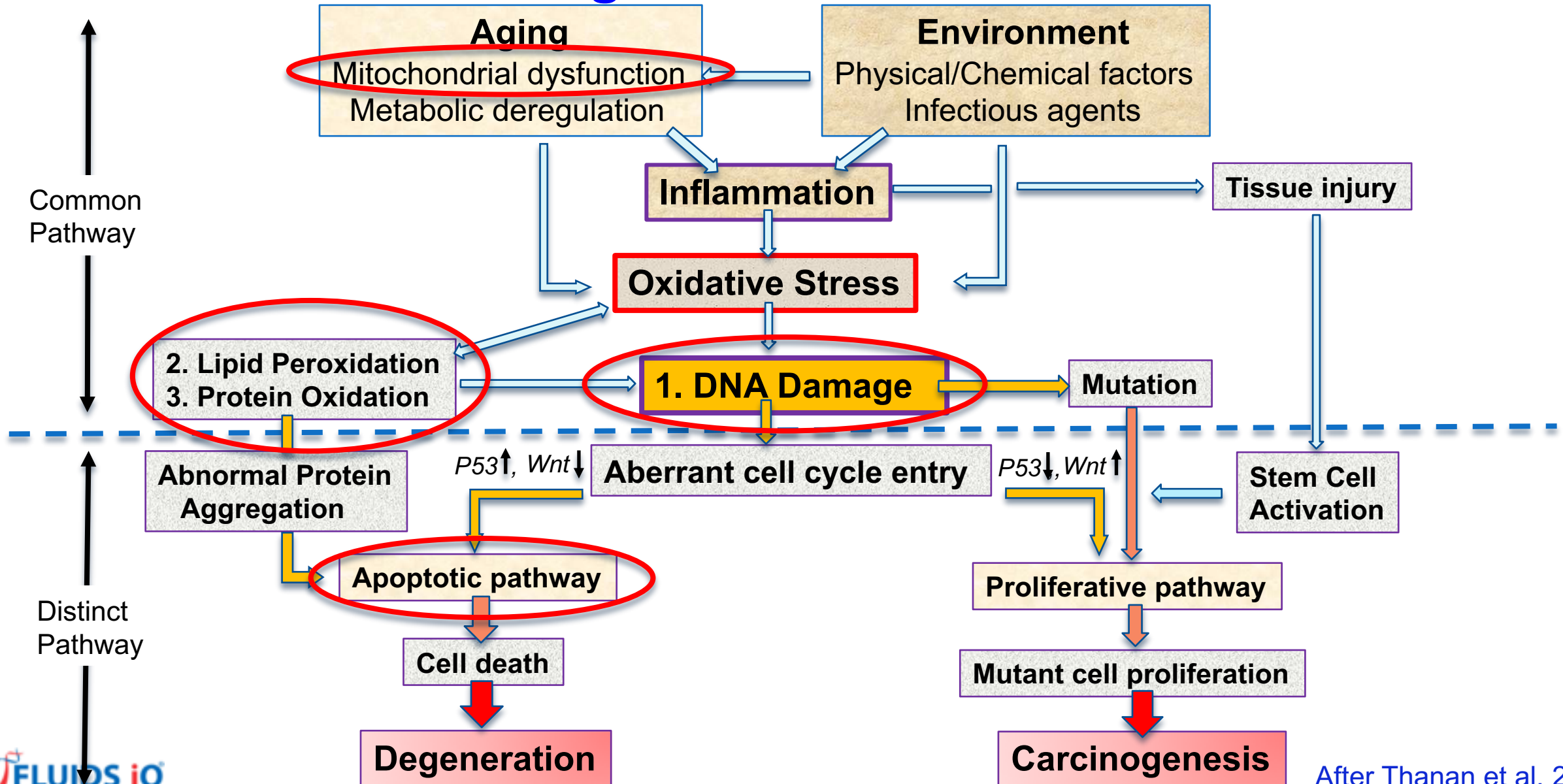




# OS, Age-Related Diseases & Relevant Biomarkers



# OS Roles in Degenerative Diseases & Cancer



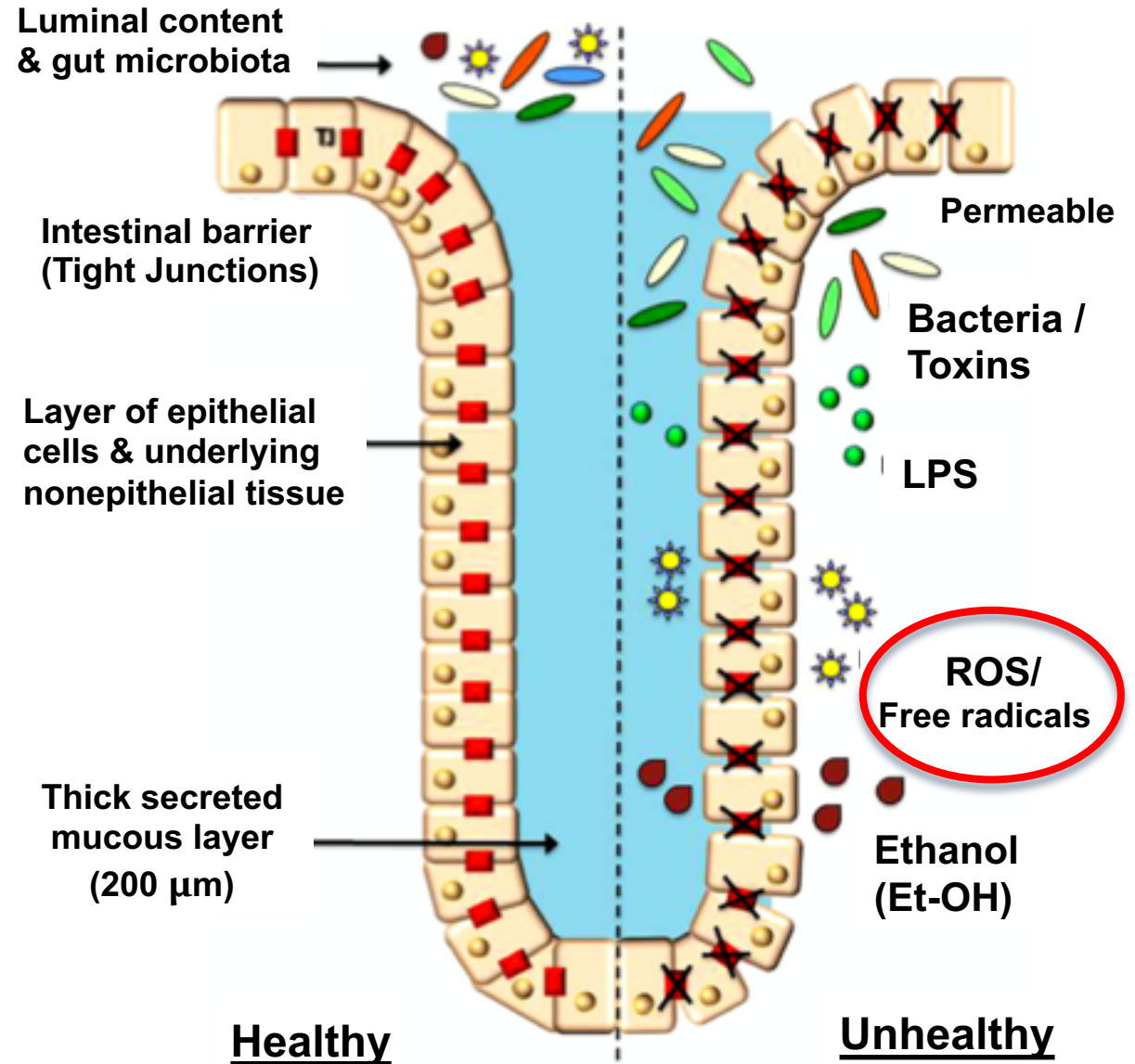
# Intestinal Barrier & protection against OS

OS causes increased carbonylation of cellular proteins, including the actin and microtubule cytoskeletons.

- Protein Carbonylation is irreversible oxidative damage = loss of protein function
- Indicator of severe oxidative damage & disease-derived protein dysfunction

Results: Loss of TJ integrity and increased paracellular permeability

Farhadi. '06



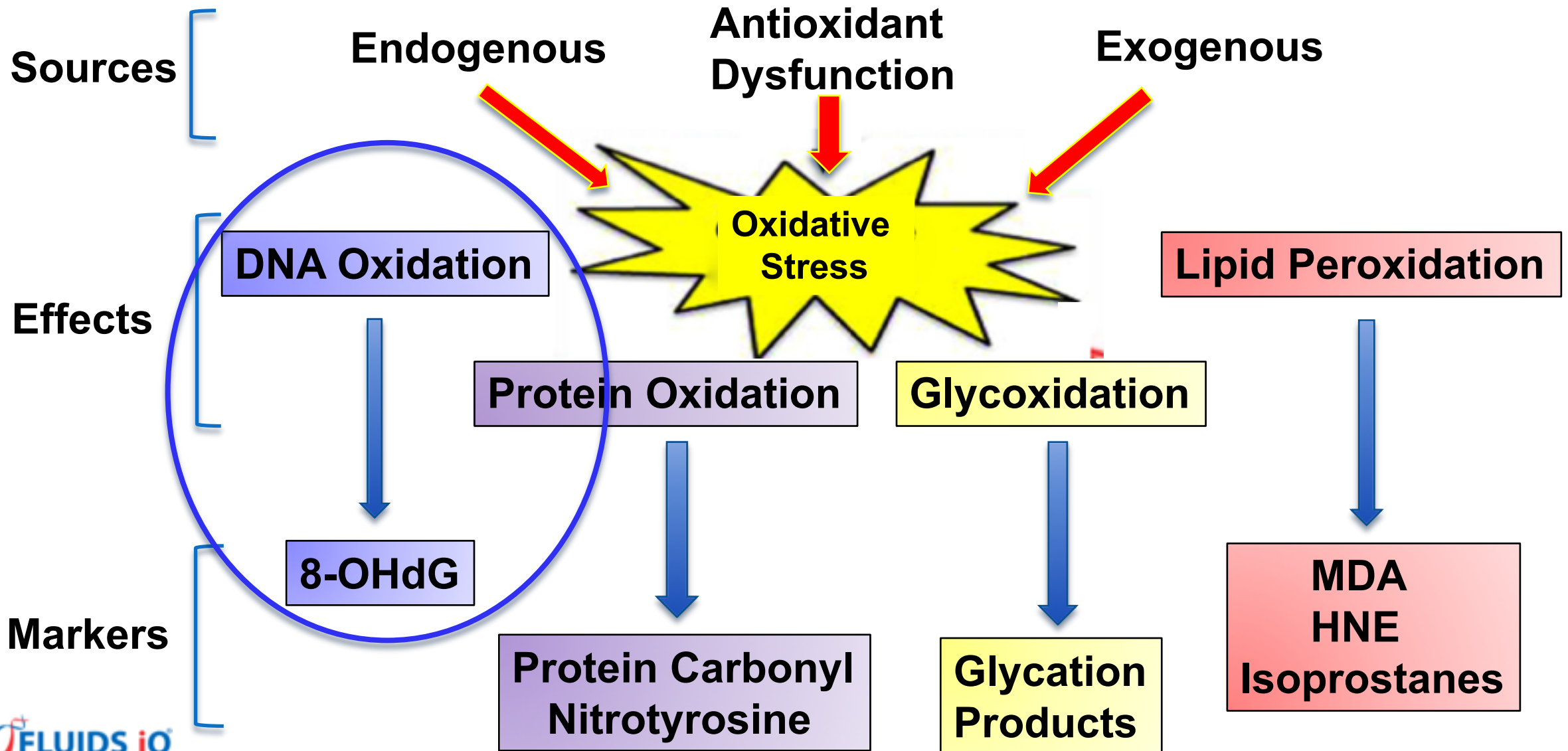
# Frequently Used Biomarkers of Oxidative Stress

Marker or Type of Damage	Sample Type				
	Cells	Tissues	Blood	Urine	Other
<i>DNA / RNA Damage</i>					
8-hydroxyguanosine (8-OHG)	X	X	X	X	Cerebrospinal fluid
*8-hydroxydeoxyguanosine (8-OHdG)	X	X	X	X	
Abasic (AP) sites	X	X			
BPDE DNA Adduct	X	X			
Double-strand DNA breaks	X				
Comet Assay (general DNA damage)	X				
UV DNA Damage (CPD, 6-4PP)	X				
<i>Lipid Peroxidation</i>					
4-Hydroxynonenal (4-HNE)	X	X	X		
8-iso-Prostaglandin F2alpha (8-isoprostane)	X	X	X	X	
Malondialdehyde (MDA)	X	X	X	X	
TBARS	X	X	X	X	

Marker or Type of Damage	Sample Type				
	Cells	Tissues	Blood	Urine	Other
<i>Protein Oxidation / Nitration</i>					
*Protein Carbonyl Content (PCC)	X	X	X		
3-Nitrotyrosine	X	X	X		
Advanced Glycation End Products (AGE)	X	X	X		
Advanced Oxidation Protein Products (AOPP)	X	X	X		
BPDE Protein Adduct	X	X	X		
<i>Reactive Oxygen Species</i>					
Universal ROS / RNS	X	X	X	X	
Hydrogen Peroxide	X	X	X	X	
Nitric Oxide	X	X	X	X	
<i>Antioxidants</i>					
Catalase	X	X	X		
Glutathione	X	X	X	X	
Superoxide Dismutase	X	X	X		
Oxygen Radical Antioxidant Capacity (ORAC)	X	X	X	X	Food samples
Hydroxyl Radical Antioxidant Capacity (HORAC)	X	X	X	X	Food samples
Total Antioxidant Capacity (TAC)	X	X	X	X	Food samples
Cell-Based Exogenous Antioxidant Assay					Food samples, Antioxidants

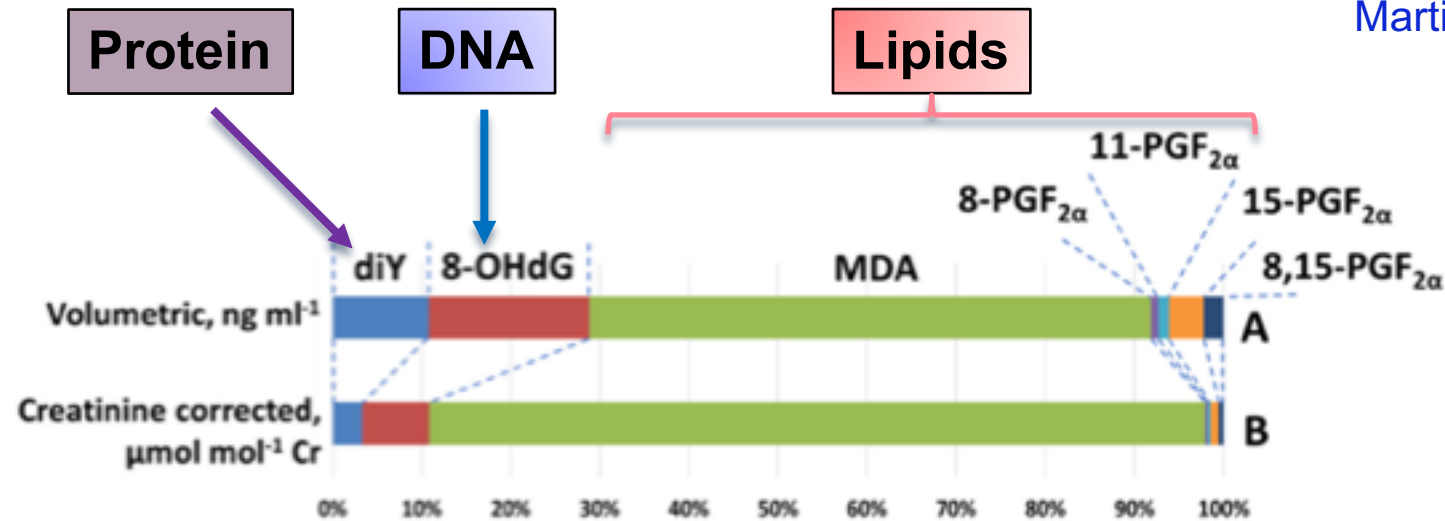


# Oxidative Stress - Cellular Damage - Markers



# Profiles of OS Biomarkers in Spot Urine samples

Martinez-Moral: Environ Int-2019



- Diurnal & inter-individual variability in OS status in healthy individuals

- **8-OHdG:**

- Highest test/retest reliability (ICC = 0.96)
- Most reproducible measurements, with lowest variability (low CV)

**Most suitable biomarker of OS Biomarkers in SPOT urine samples**

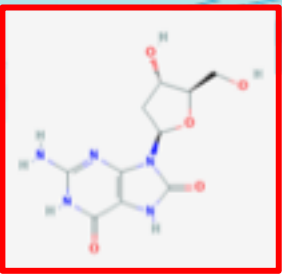


# The DNA Oxidative Damage Test

## 8-Hydroxy-2'-deoxyguanosine: **8-OHdG**

- Biomarker of **cumulative** cellular oxidative stress
- Quantitatively assesses ongoing oxidative damage
- Monitors effects of antioxidant therapies & detoxification
- Simple collection: Dried urine

# 8-Hydroxy-2'-deoxyguanosine: 8-OHdG



- Response to normal metabolic processes & environmental factors
  - Produced by DNA oxidative damage due to reactive O<sub>2</sub> & N<sub>2</sub> species<sup>1</sup>
- Increased 8-OHdG levels associated with many dysfunctions/diseases:
  - Aging process, COPD<sup>2</sup>, cancer, diabetes, hypertension<sup>3</sup> and others
- DNA oxidative damage also affects cell Lipid & Protein function

**Cellular 8-OHdG Concentration:  
Established Marker of Oxidative Stress**

# OS & ROS-Induced Urinary 8-OHdG Elevation

## Association with Pathological Processes



- Inflammatory Bowel Disease
- Metabolic Syndrome
- Pancreatitis
- Hyperglycemia
- Alzheimer's Disease
- Chronic Hepatitis
- Atopic Dermatitis

- Parkinson's Disease
- Alzheimer's Disease
- Rheumatoid Arthritis
- Huntington's Disease
- Diabetic Nephropathy
- Diabetic Retinopathy
- Cystic Fibrosis

# Elevated Urinary 8-OHdG

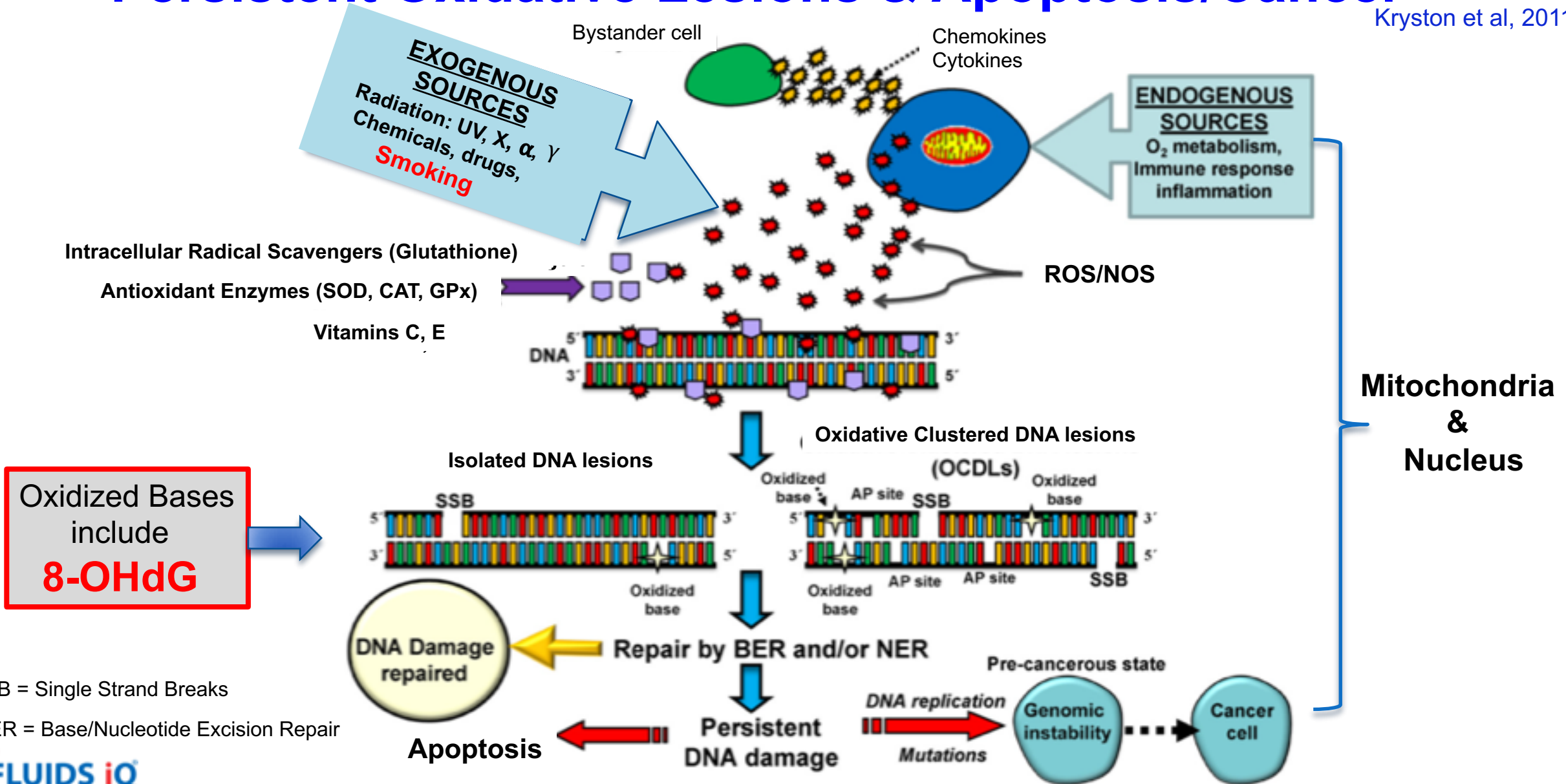
## Exposure to Environmental Factors

- **Cigarette smoke**
- **Diesel exhaust**
- **Asbestos**
- **Toxic metals**
- **Radon (indoors)**

- **Fumes**
  - Chromium, Manganese, Vanadium
- **Styrene**
- **Toluene**
- **Xylene**
- **Benzene**

# Persistent Oxidative Lesions & Apoptosis/Cancer

Kryston et al, 2011





# Antioxidants

Endogenous

Exogenous

Non-Enzymatic

Enzymatic

Glutathione (GSH)

Thioredoxin (TRX)

Ubiquinone

Uric Acid

Lipoic Acid

Bilirubin

Catalase (CAT)

Superoxide dismutase (SOD)

Glutathione peroxidase (GPx)

Glutathione reductase (GR)

Vit A, C, E, K

B-Carotene

Glutathione

Polyphenols

Malondialdehyde (MDA)

Ubiquinone (Coenzyme-Q10)

Phenolic Acids

Flavonoids

Flavones

Flavanols

Quercetin

Flavanones

Isoflavanones

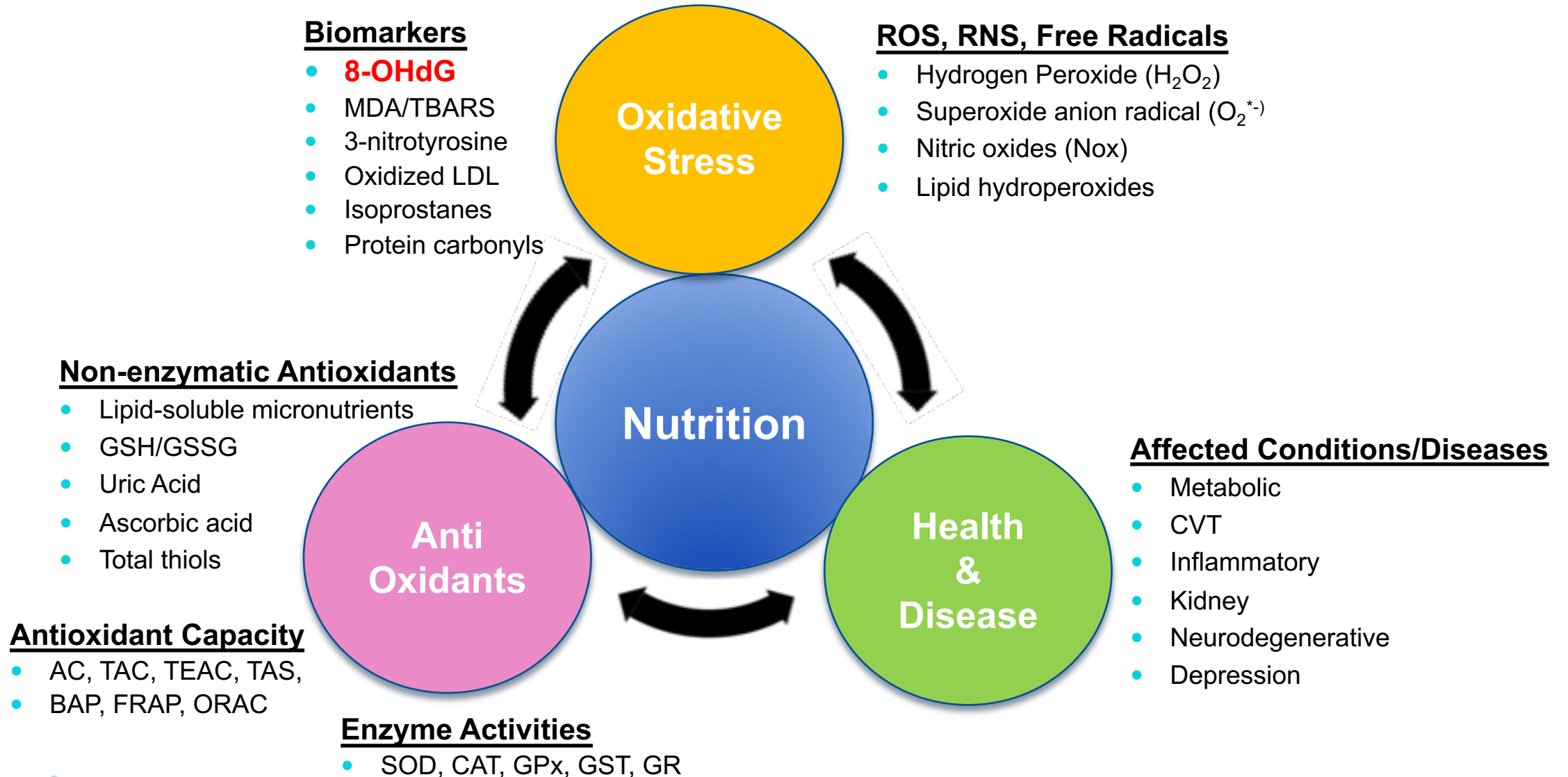
Anthocyanidins

Resveratrol

Curcumin



# Interaction of Nutrition, Antioxidants, OS & Health Status





**Biomarkers:  
Intestinal Permeability & Metabolic Wellness**

*Dr Aron Gonshor: PhD, DDS, FRCD(C), FAO*

*February 10, 2021*

