Webinar PAIS, metabolome, and probiotics

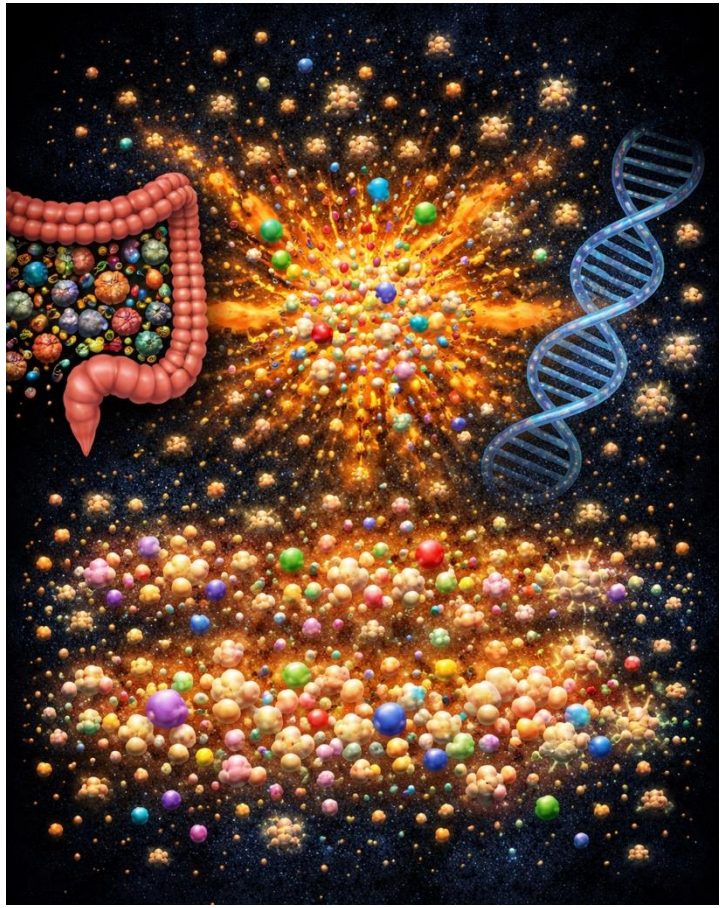
An approach to use the metabolome in the clinical practice

19-3-2026

Roy Montijn, Mariya Petrova, Dennis Zeilstra

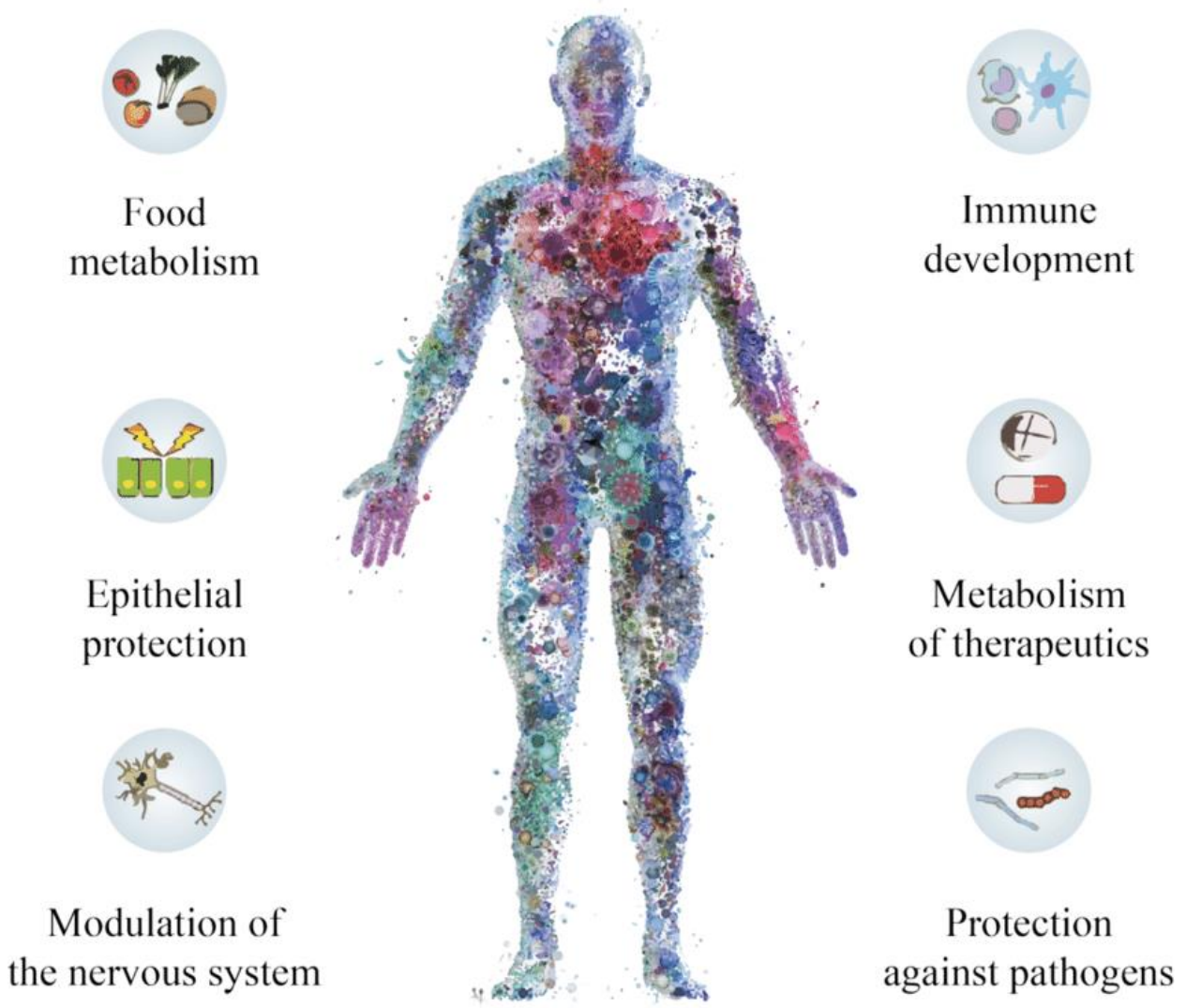
Microbiome-human interaction:

often via metabolites



Role of the Gut Microbiome

- Food metabolism - SCFA, vitamins, neurotransmitters
- Protective functions - bacteriocins, competition,
- Development of structural functions - villi, crypts, tight junctions
- Immune-modulating functions



Opstal, E. J. van et al.Science.349,.1172–1173.(2015)

Gut-Organ Axis

Main classes of beneficial gut metabolites

Short-chain fatty acids (SCFAs)
- Acetate, Propionate, Butyrate

Amino acid-derived metabolites
- Indoles, Phenols, Amines, Branched fatty acids

Lipid-derived metabolites
- Glycerol derivatives, Choline metabolites (e.g., TMAO)

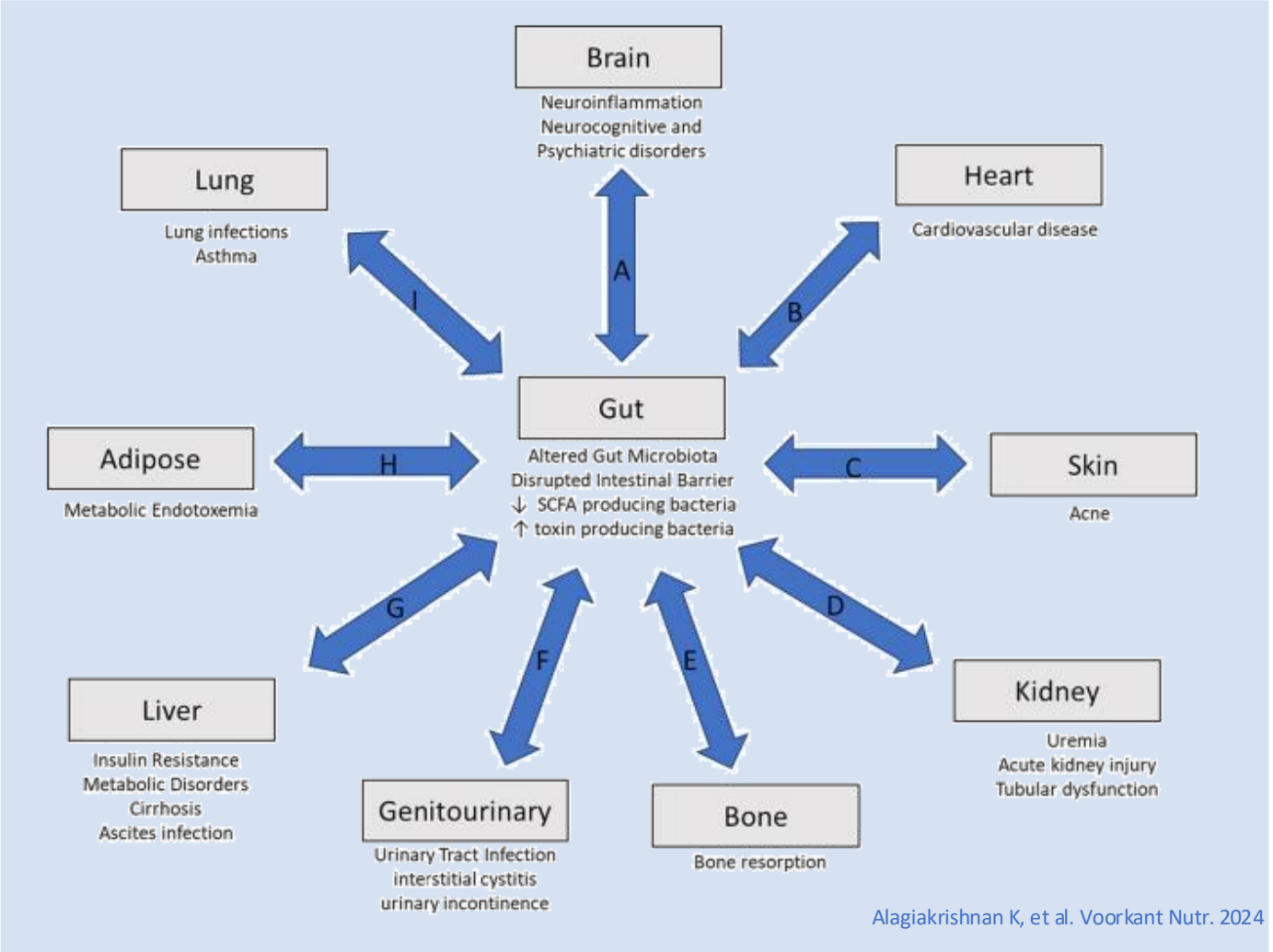
Vitamins and cofactors
- B vitamins (e.g., B12, Biotin, Folic acid), K vitamin

Polyphenol metabolites

Gas metabolites
- Hydrogen, methane, hydrogen sulfide

Neuroactive substances
- GABA, serotonin precursors, tryptamine

Secondary bile acids

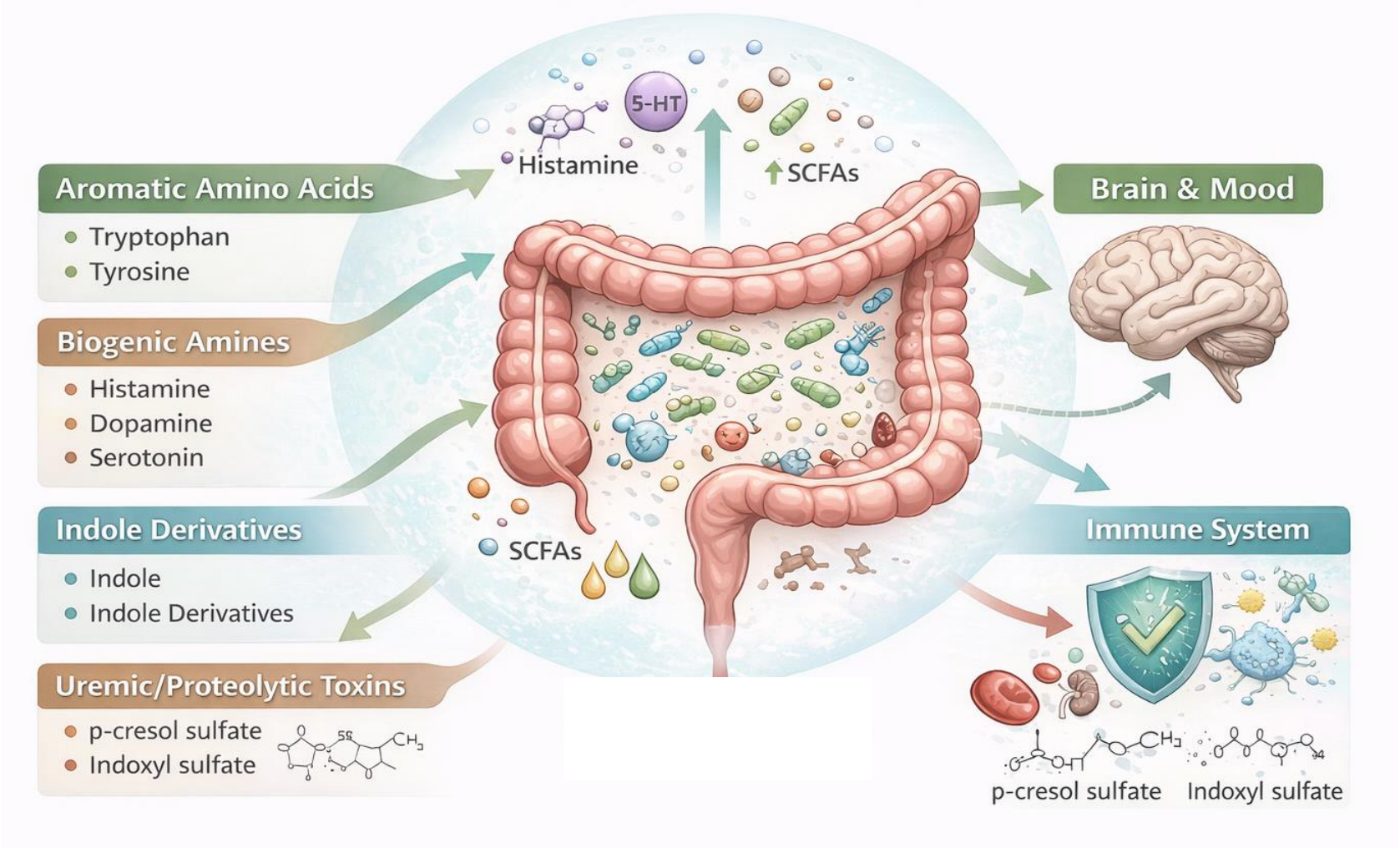


Alagiakrishnan K, et al. Voorkant Nutr. 2024

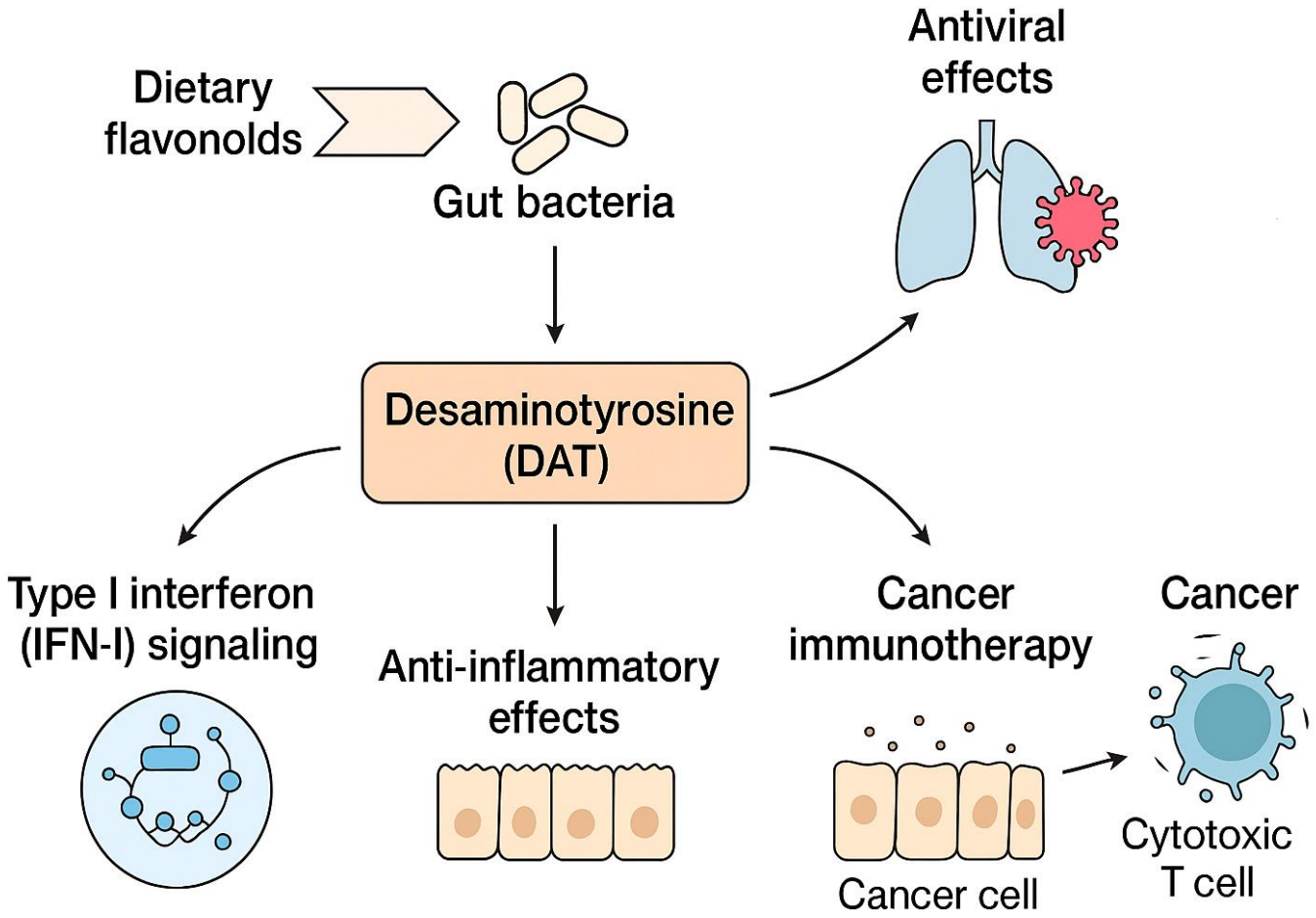
Key & measurable microbial metabolites

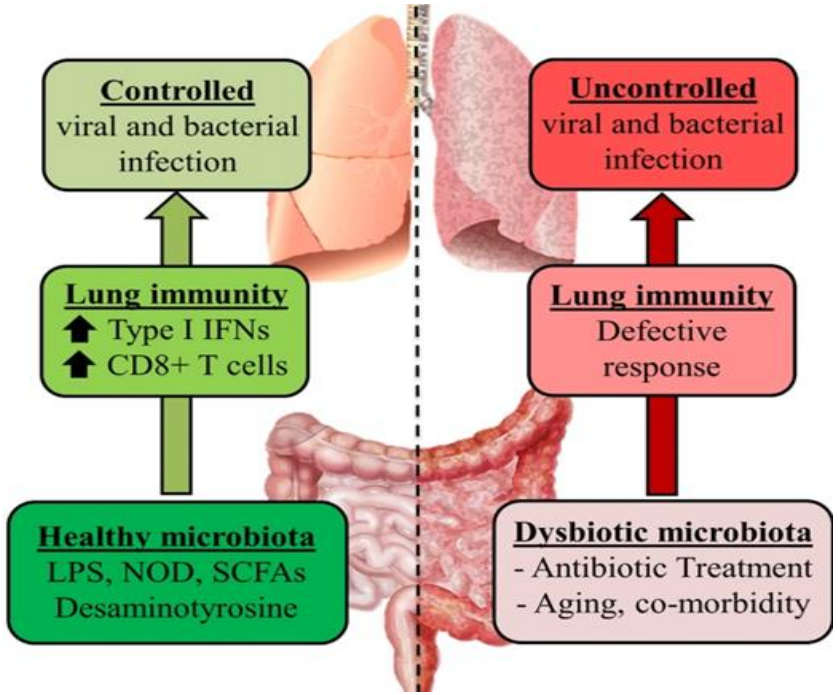
The microbiota influences host metabolism

- Aromatic amino acids (tryptophan, tyrosine)
- Biogenic amines (histamine, dopamine, serotonin)
- Indole derivatives
- Uremic/proteolytic toxins (p-cresol sulfate, indoxyl sulfate)
- Short-chain fatty acids (SCFAs)
- Secondary bile acids
- …
- …



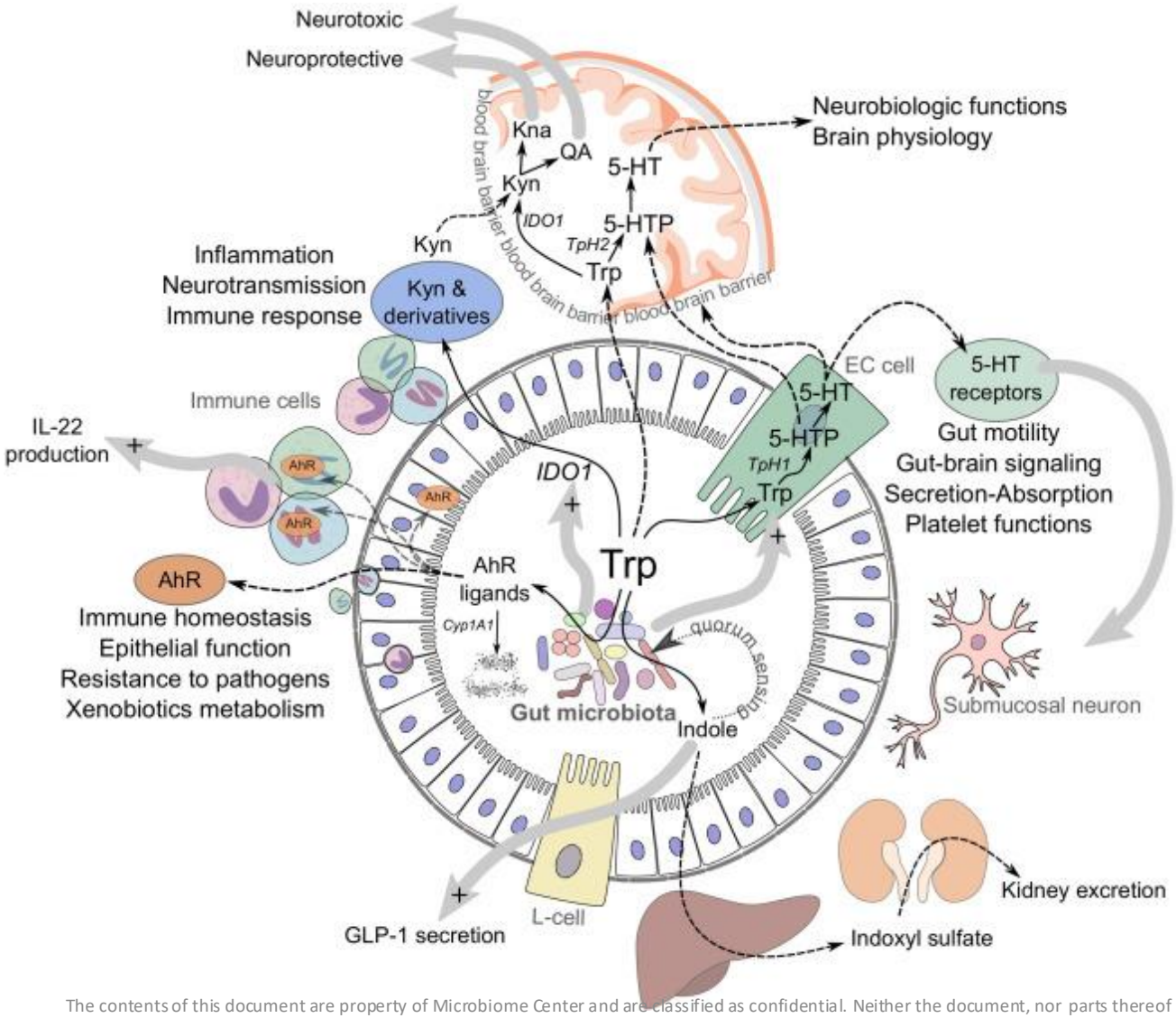
Example - Gut-Lung Axis





Göttert S, *et al*. Nat Commun 2025
Sencio *et al*. Mucosal Immunol 2021
Steed *et al*. Science 2017
Rosshart *et al*. Cell 2017
Ichinohe *et al*. PNAS 2011

Tryptophane Metabolism



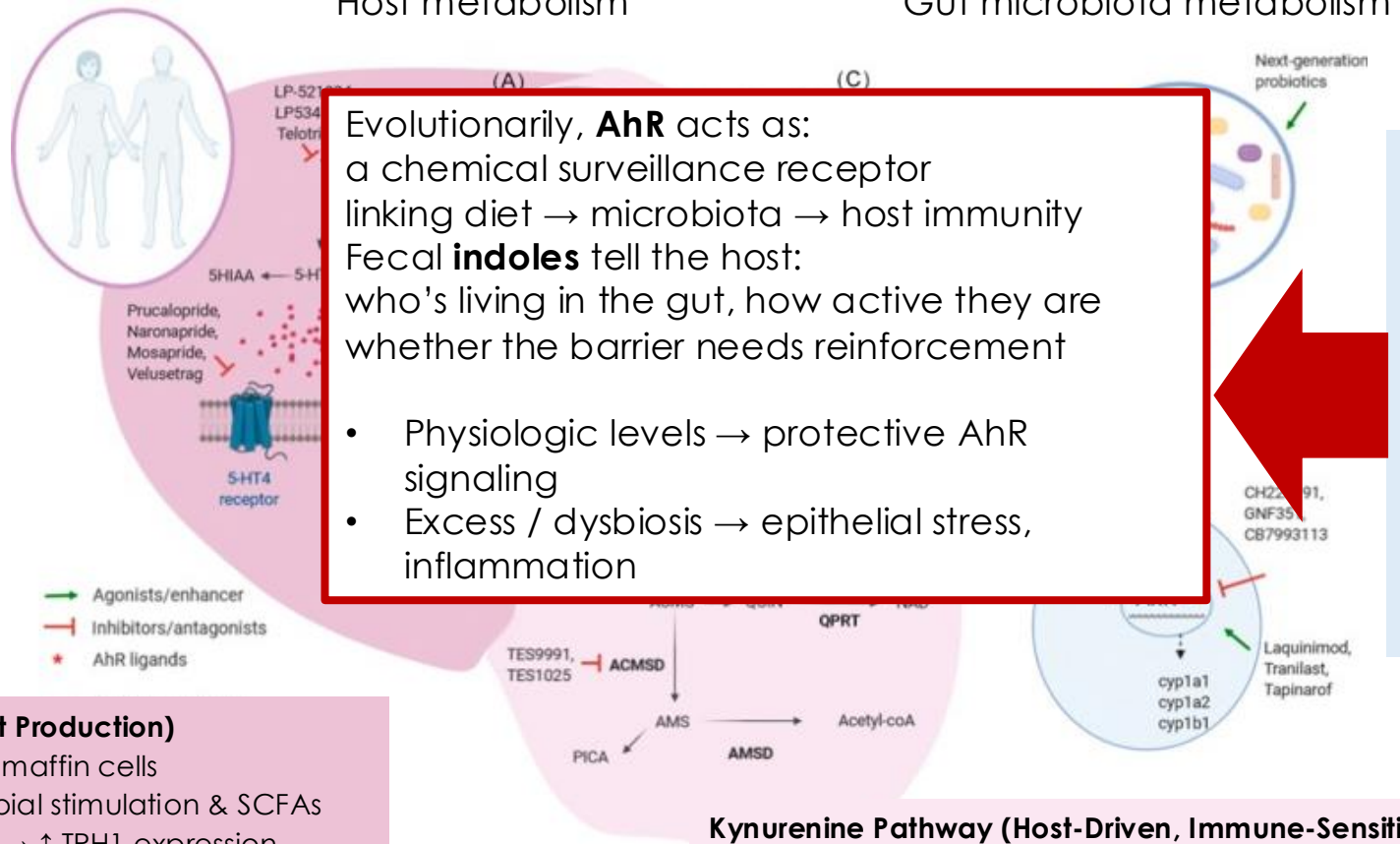
- Serotonin pathway (~5%) – indirect microbiota involvement
- Kynurenine pathway (~90%) – indirect microbiota involvement
- Indole pathway (~5%) (AhR signaling) – direct microbiota involvement

Agus et al., Cell Host and Microbe 2018

Zoom in to the Tryptophane metabolism

Host metabolism

Gut microbiota metabolism



Evolutionarily, **AhR** acts as:
a chemical surveillance receptor
linking diet → microbiota → host immunity
Fecal **indoles** tell the host:
who's living in the gut, how active they are
whether the barrier needs reinforcement

- Physiologic levels → protective AhR signaling
- Excess / dysbiosis → epithelial stress, inflammation

Indole Pathway -Direct bacterial metabolism of tryptophan

- Indole (via tryptophanase)
- Tryptamine (via decarboxylases)
- Indole-3-propionic acid (IPA)
- Indole-3-aldehyde (IAld)

Functions:

- Activation of Aryl hydrocarbon receptor (AhR) and IL-22 expression
- Barrier support & immune regulation
- Anti-inflammatory & antioxidant effects (IPA)

Serotonin Pathway (Host Production)

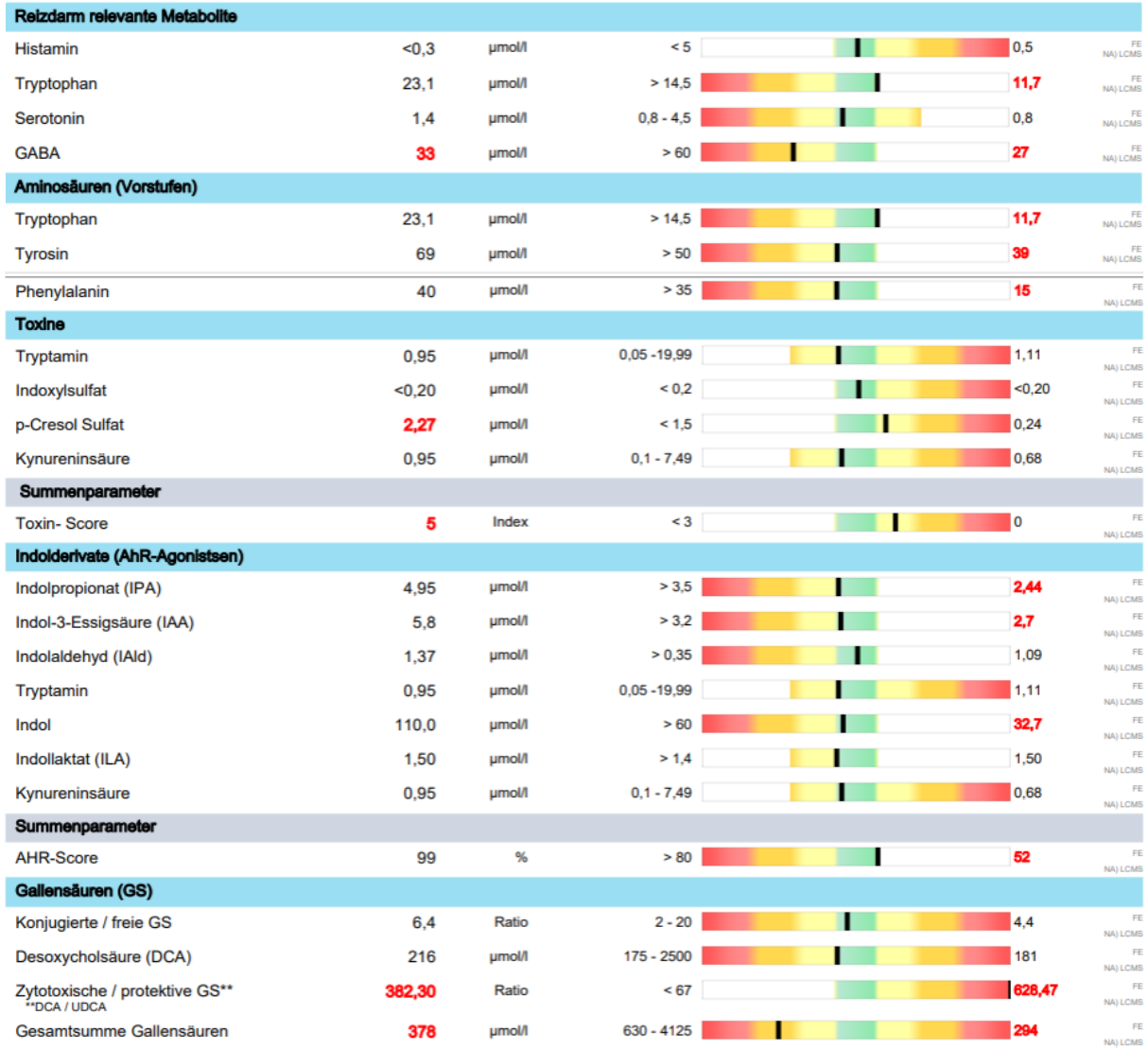
- Occurs in enterochromaffin cells
- Regulated by microbial stimulation & SCFAs (especially butyrate) → ↑ TPH1 expression
- Low-butyrate states leads to ↓ Gut serotonin, Altered motility and gut function

Kynurenine Pathway (Host-Driven, Immune-Sensitive) - Dominant route of tryptophan metabolism.

- Microbiota influence this pathway indirectly via immune signaling.
- Activated by inflammation (↑ IFN-γ, TNF-α)
- Induces IDO1
- Tryptophan → kynurenine
- Effects of dysbiosis:↓ Circulating tryptophan↓ Serotonin availability↑ Neuroactive kynurenines

Modoux et al., Trends in Pharmacological Science 2021

The Biovis 'microbolome' measurement

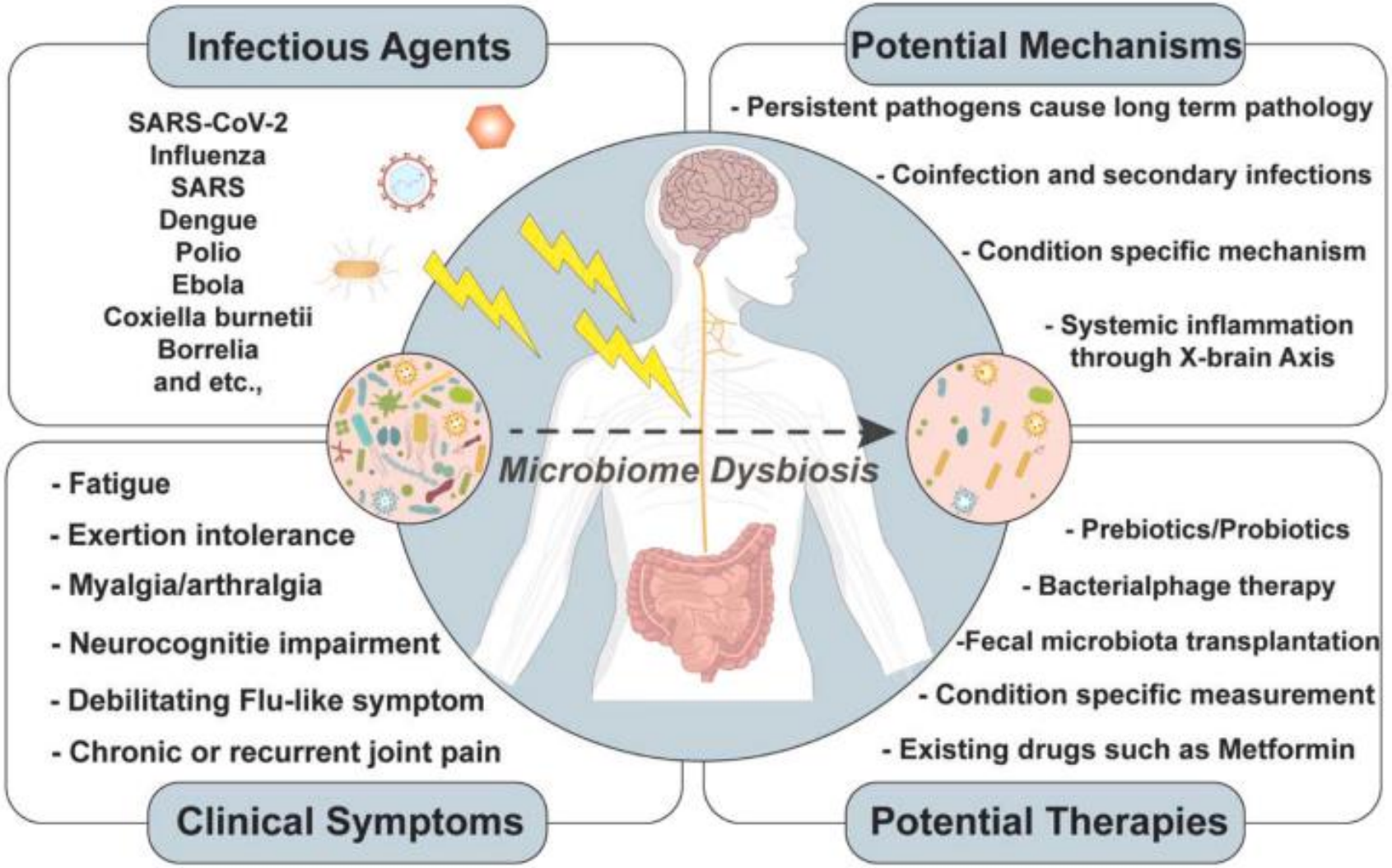


Reizdarm relevante Metabolite				
Histamin	<0,3	µmol/l	< 5	0,5
Tryptophan	23,1	µmol/l	> 14,5	11,7
Serotonin	1,4	µmol/l	0,8 - 4,5	0,8
GABA	33	µmol/l	> 60	27
Aminosäuren (Vorstufen)				
Tryptophan	23,1	µmol/l	> 14,5	11,7
Tyrosin	69	µmol/l	> 50	39
Phenylalanin	40	µmol/l	> 35	15
Toxine				
Tryptamin	0,95	µmol/l	0,05 -19,99	1,11
Indoxylsulfat	<0,20	µmol/l	< 0,2	<0,20
p-Cresol Sulfat	2,27	µmol/l	< 1,5	0,24
Kynureninsäure	0,95	µmol/l	0,1 - 7,49	0,68
Summenparameter				
Toxin- Score	5	Index	< 3	0
Indolderivate (AhR-Agonistsen)				
Indolpropionat (IPA)	4,95	µmol/l	> 3,5	2,44
Indol-3-Essigsäure (IAA)	5,8	µmol/l	> 3,2	2,7
Indolaldehyd (IAld)	1,37	µmol/l	> 0,35	1,09
Tryptamin	0,95	µmol/l	0,05 -19,99	1,11
Indol	110,0	µmol/l	> 60	32,7
Indollaktat (ILA)	1,50	µmol/l	> 1,4	1,50
Kynureninsäure	0,95	µmol/l	0,1 - 7,49	0,68
Summenparameter				
AHR-Score	99	%	> 80	52
Gallensäuren (GS)				
Konjugierte / freie GS	6,4	Ratio	2 - 20	4,4
Desoxycholsäure (DCA)	216	µmol/l	175 - 2500	181
Zytotoxische / protektive GS** **DCA / UDCA	382,30	Ratio	< 67	628,47
Gesamtsumme Gallensäuren	378	µmol/l	630 - 4125	294

1. **Tryptophan Metabolism**
   - Serotonin pathway – Serotonin
   - Kynurenine pathway – Kynurenine
   - Indole pathway- indole, IPA, IAA, ILA
2. **Tyrosine Metabolism**
   - Tyrosine
3. **GABA**
4. **Proteolytic Toxins**
   - Tryptamine
   - p-cresol sulfate
   - Indoxyl sulfate
5. **Histamine**
6. **Bile acid metabolism**
7. **Phenylalanine Metabolism**

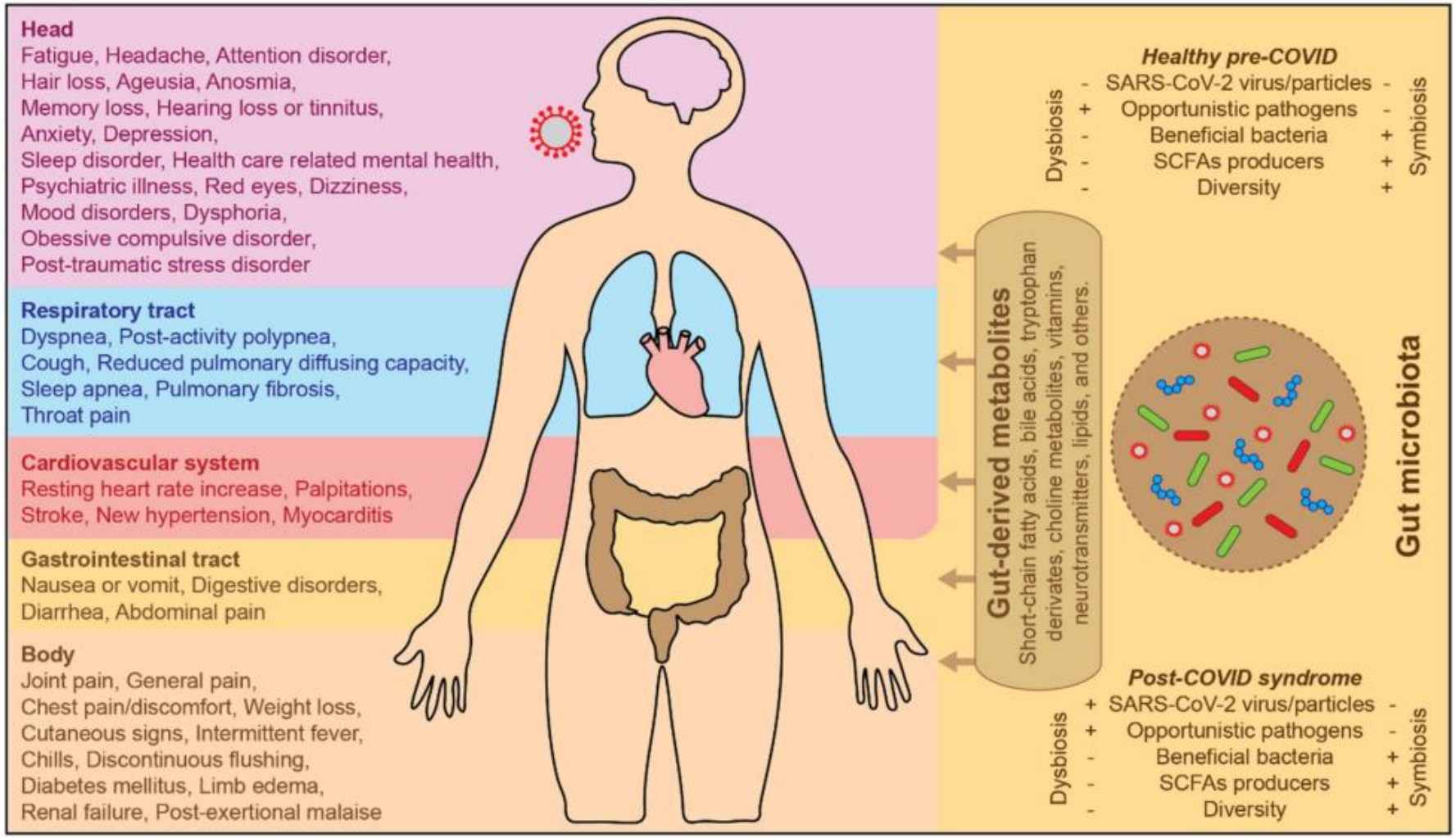
Post-Acute Infectious Syndrome (PAIS) and the **Gut microbiome**

Dysbiosis and PAIS



Guo C, et al. The microbiome in post-acute infection syndrome. Comput Struct Biotechnol J. 2023

Gut microbe–host interactions in post-COVID syndrome: a debilitating or restorative partnership?



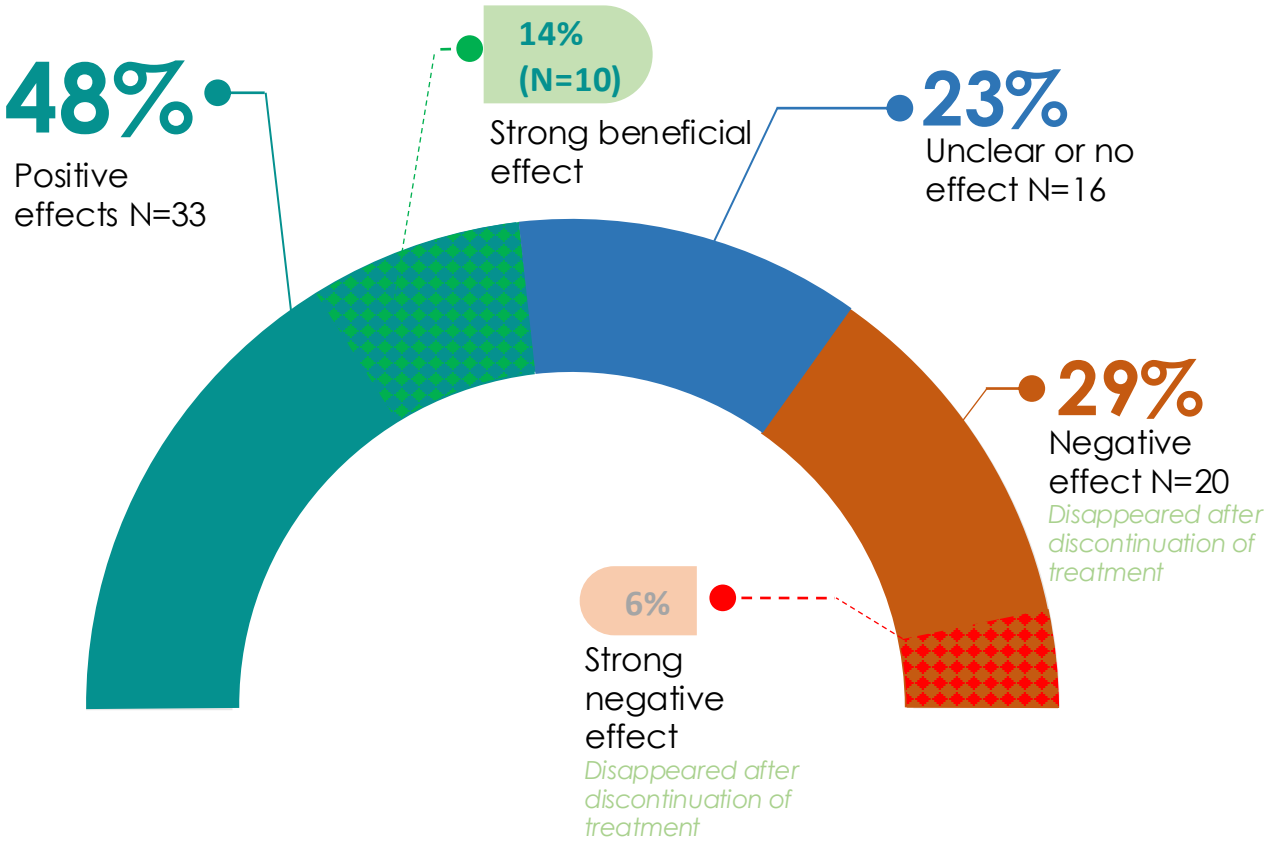
T.P. M. Scheithauer, R.C. Montijn, & A.Mieremet. Gut Microbes. 2024

Pilot with Q-fever Fatigue Syndrome (QFS) patients who have been ill for an average of 16 years

Microbiome Center

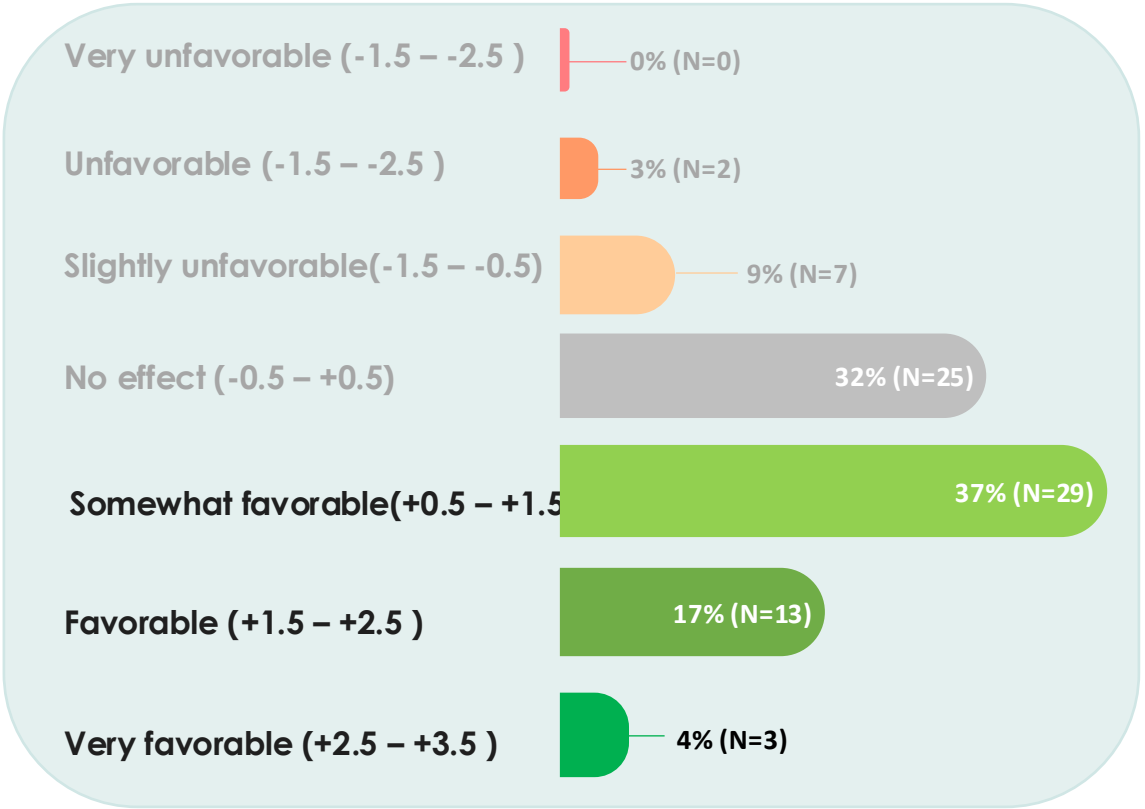
48% see improvement in general well-being(n=69)

"How are you doing (in the past week(s)?"

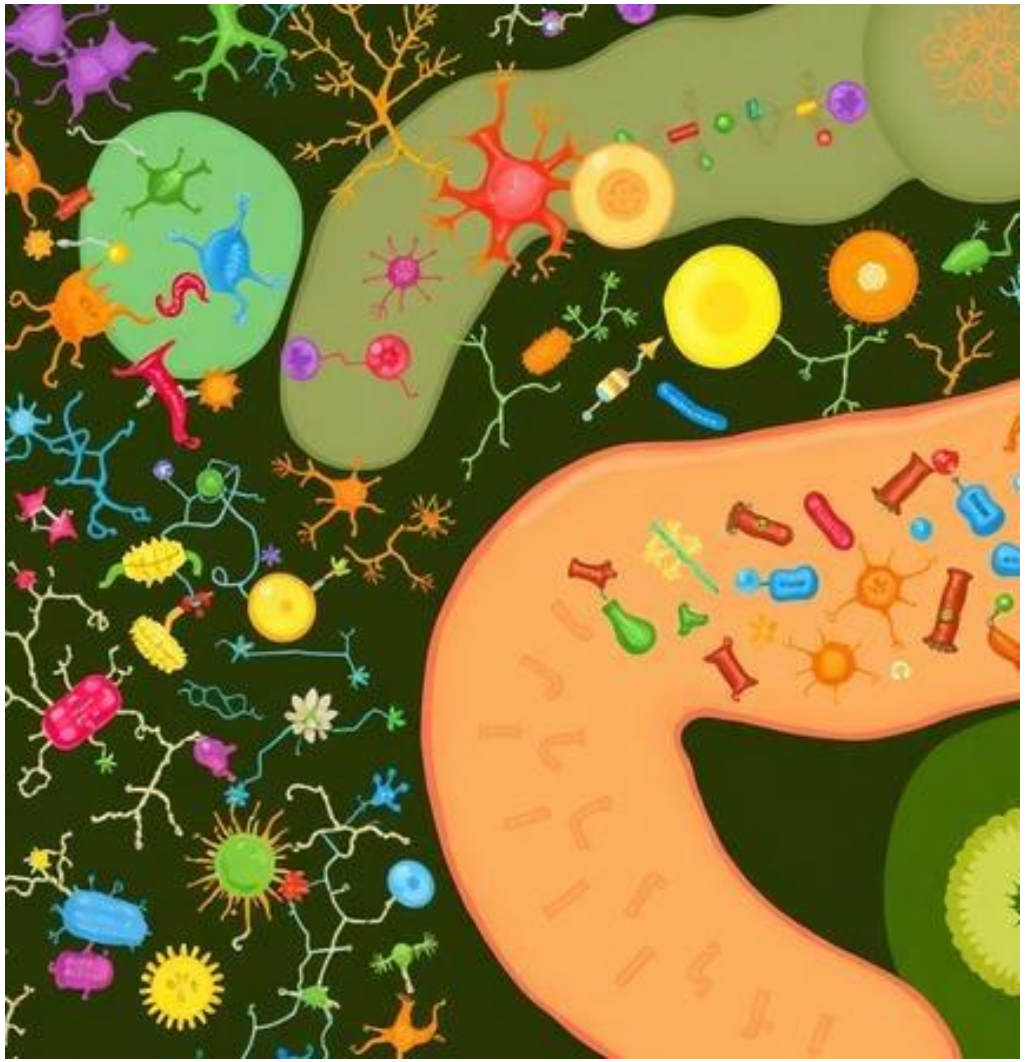


57% favourable to very favourable effect on combined complaints (n=79)

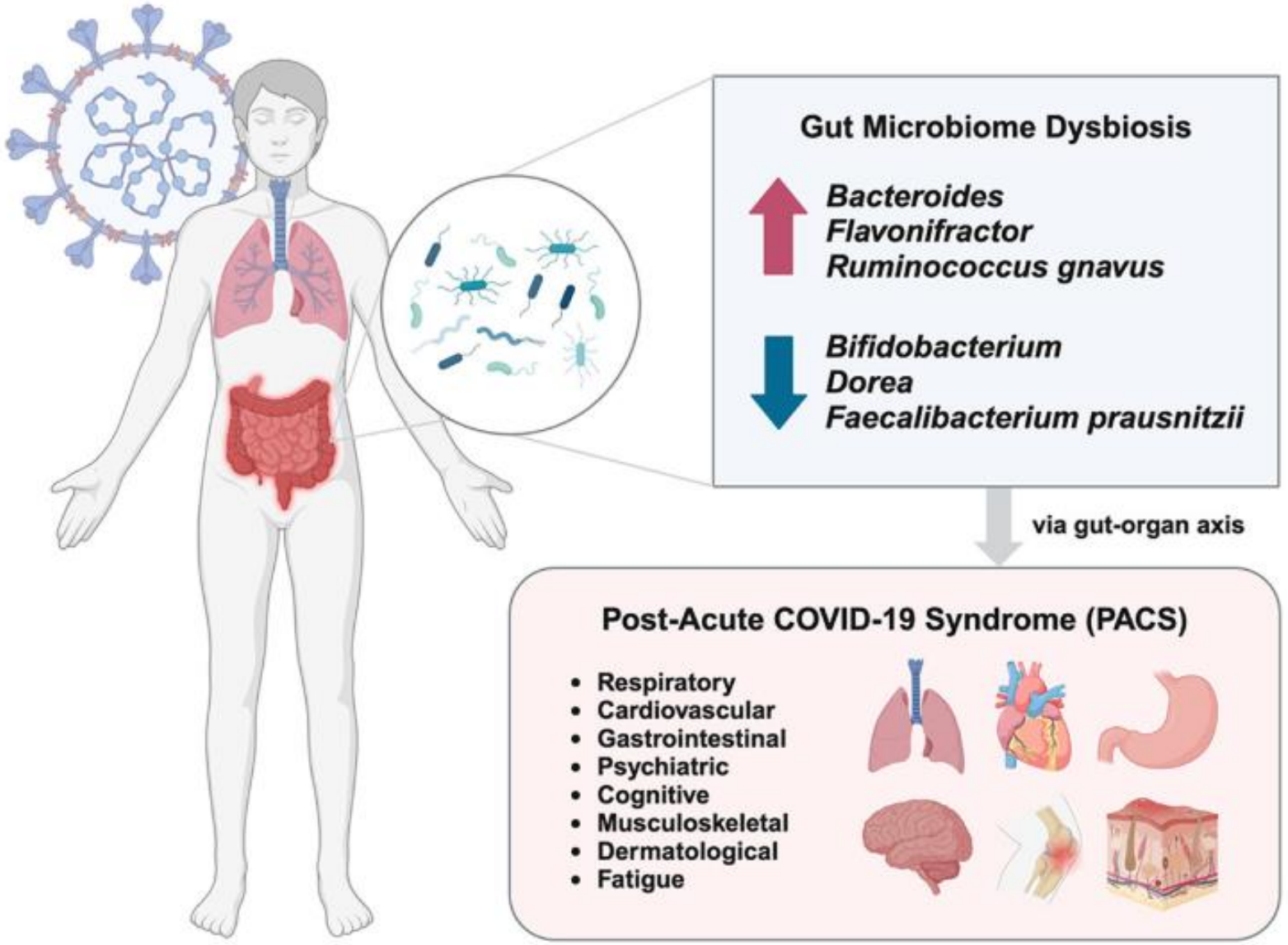
Categorized as a score from -4 (strongly negative) to +4 (strongly positive)



PAIS and the **Metabolome**



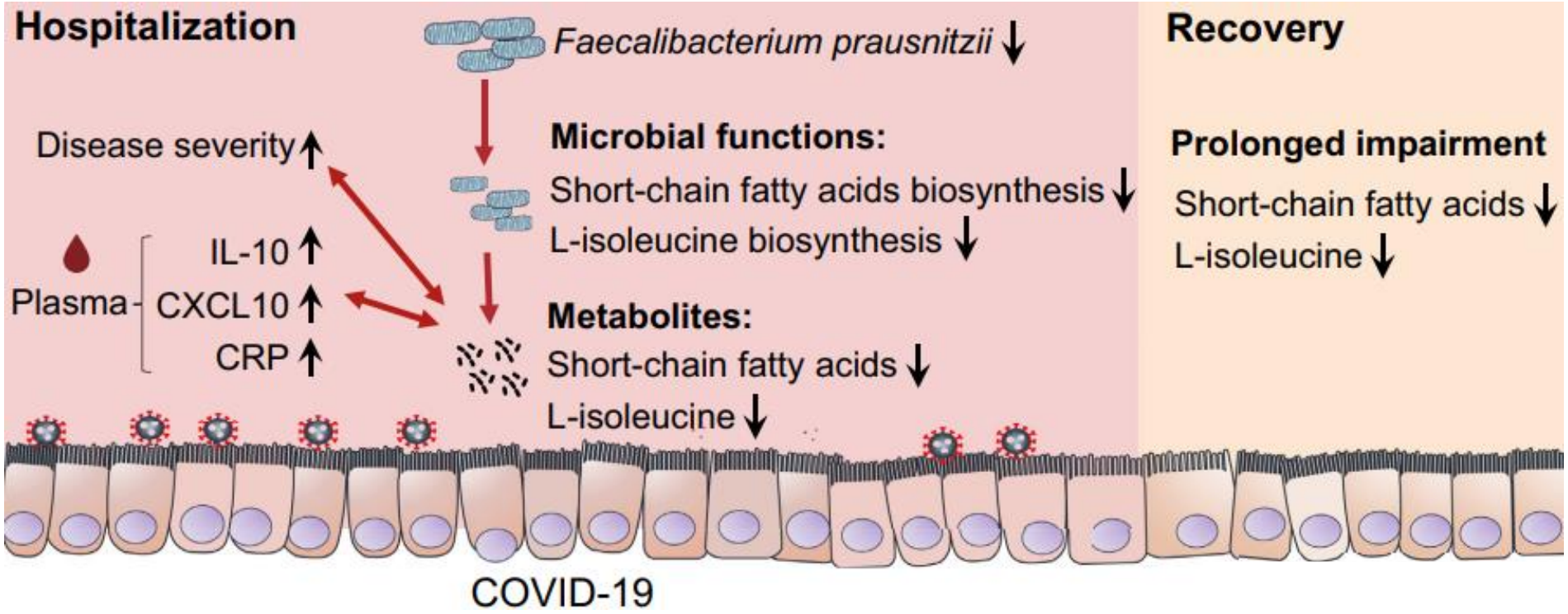
Long COVID and Dysbiosis



Lau RI, Su Q, Ng SC. Long COVID and gut microbiome: insights into pathogenesis and therapeutics. Gut Microbes. 2025

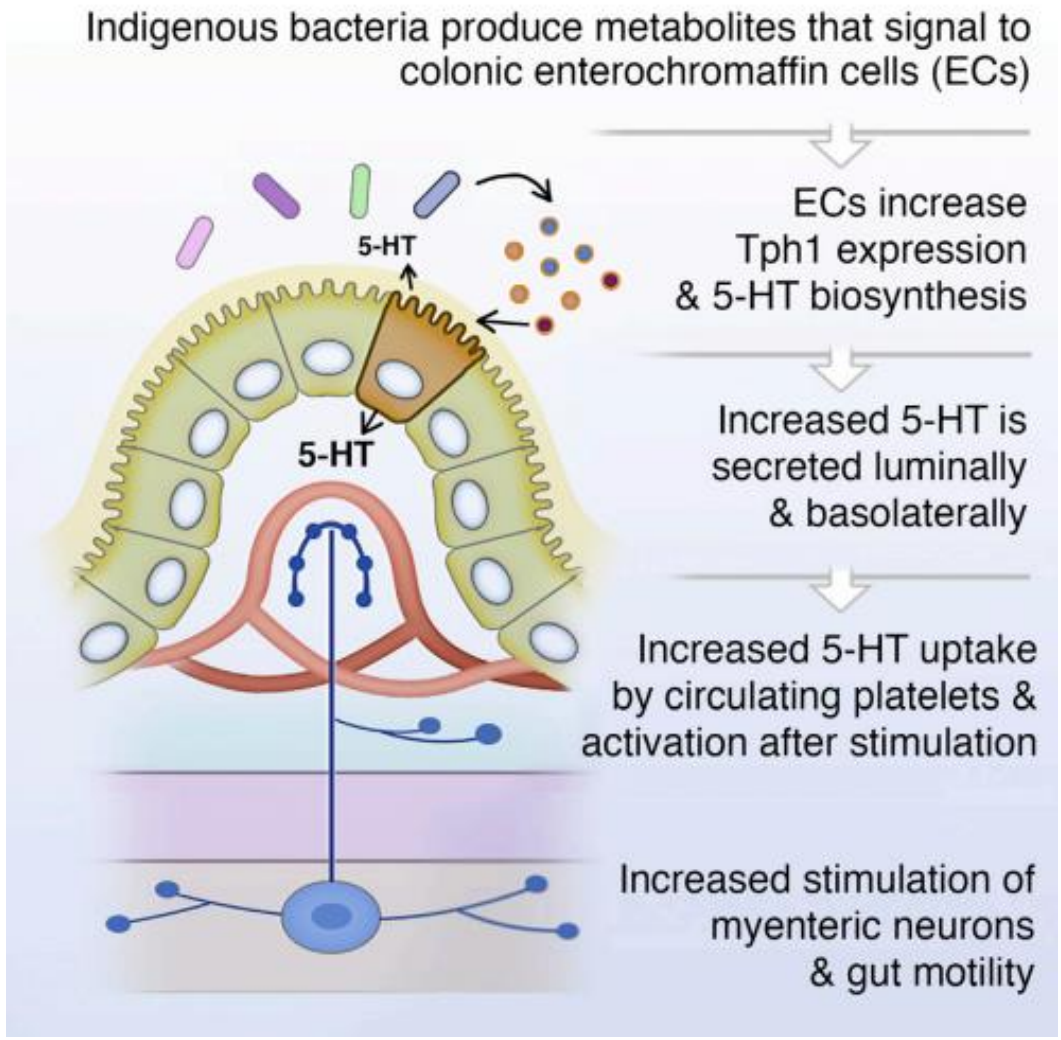
Long COVID and Metabolites Production

Production of SCFA and L-Isoleucine is low in Long-Covid



Fen Zhang et al., Prolonged Impairment of Short-Chain Fatty Acid and L-Isoleucine Biosynthesis in Gut Microbiome in Patients With COVID-19. Gastroenterology

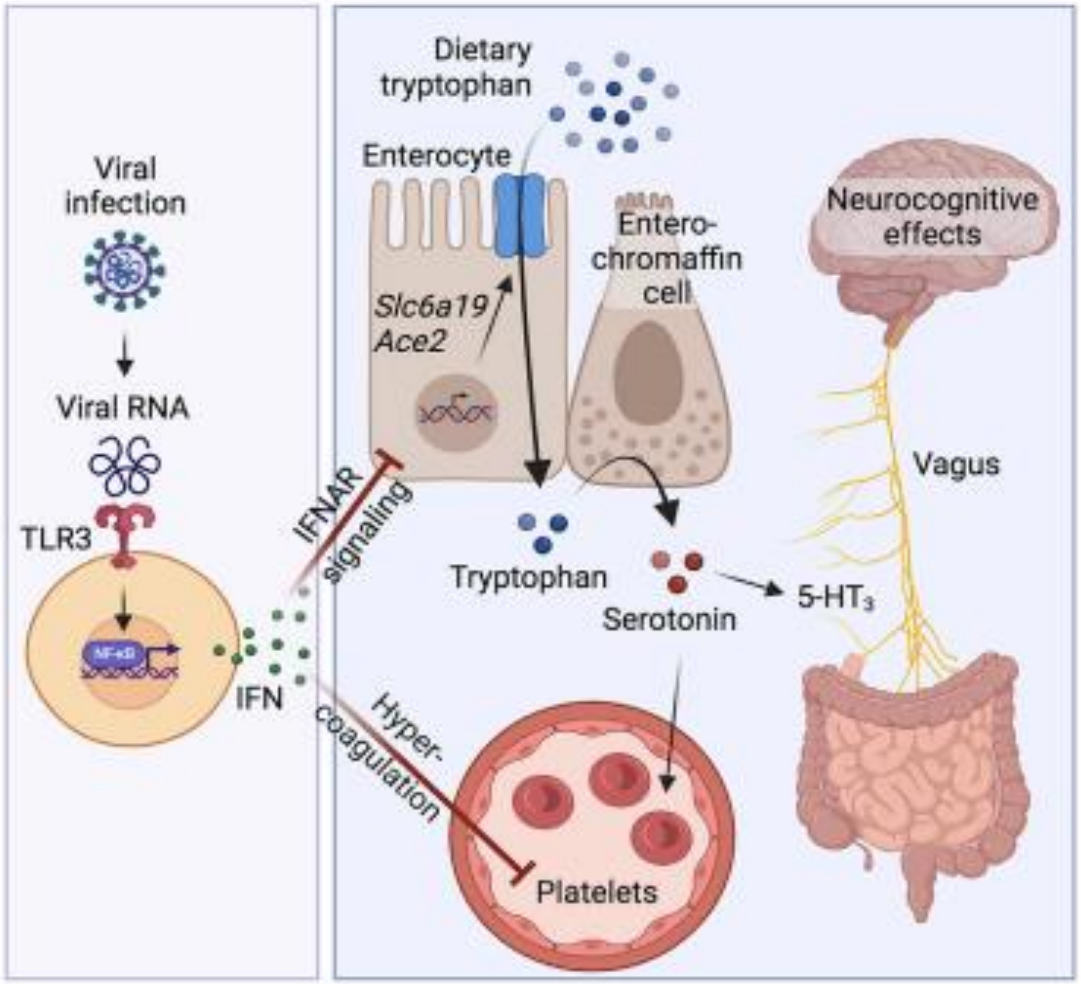
Gut bacteria regulate serotonin (5-HT) levels



- Serotonin (5-hydroxytryptamine, 5-HT), is a neurotransmitter that plays an important role in mood, cognition, learning, reward, memory and appetite, sleep, body temperature
- Gut microbes regulate levels of 5-HT in the colin and blood
- Spore-forming bacteria modulate metabolites that promote colon 5-HT biosynthesis
- Microbiota-dependent changes in 5-HT impact GI motility and hemostasis
- Altering the microbiota could improve 5-HT related disease symptoms

J.M. Yano et al., Indigenous Bacteria from the Gut Microbiota Regulate Host Serotonin Biosynthesis. 2015 Cell

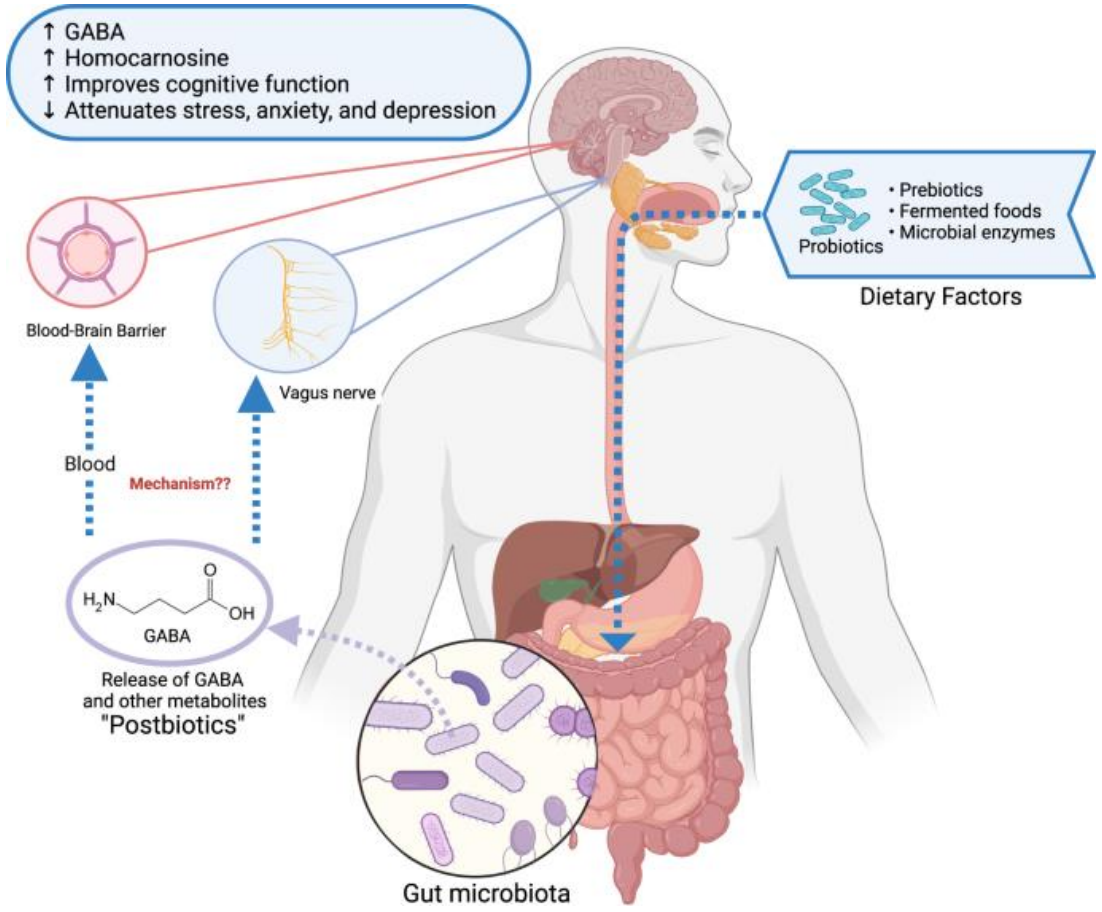
Long COVID is associated with reduced circulating serotonin levels

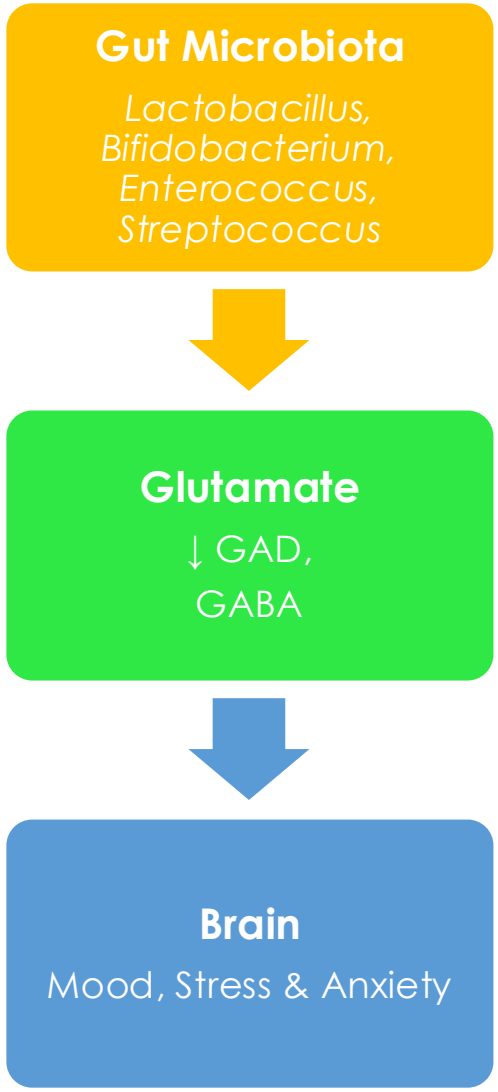


- Long Covid is associated with reduced circulating serotonin levels
- Serotonin depletion is driven by viral RNA-induced type I interferons (IFNs)
- IFNs reduce serotonin through diminished tryptophan uptake and hypercoagulability
- Peripheral serotonin deficiency impairs cognition via reduced vagal signaling

Wong et al., Serotonin reduction in post-acute sequelae of viral infection. Cell 2023

Gut Microbiota-Derived GABA





Physiological roles of intestinal GABA:

- Regulation of intestinal motility
- Modulation of secretion and absorption
- Interaction with immune cells

Braga et al., Gamma-aminobutyric acid as a potential postbiotic mediator in the gut–brain axis. npj Science and Food 2024

GABA in Long COVID

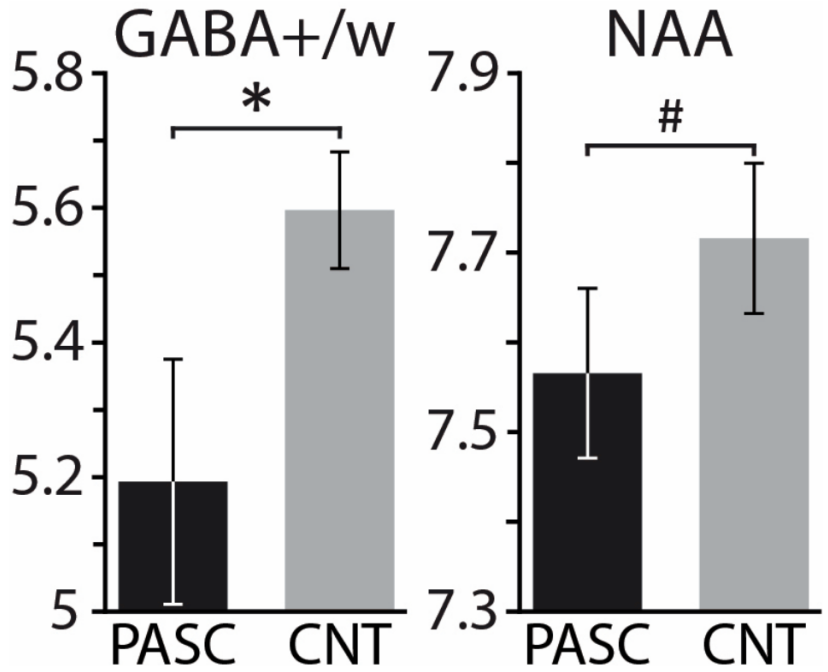


Figure 5. Group means ± standard errors of GABA+/water and NAA (N-acetylaspartate) for PASC and CNT groups. Controlling for the effect of tissue composition, biological sex, and drinking, the PASC group demonstrated lower values than CNT for both metabolites. * $p < 0.05$, # $p < 0.14$.

In Long Covid patients there is some evidence that Cortical GABA is lowered compared to healthy controls

Marinkovic, K. *et al.Brain Sciences*.13,.1666.(2023)

Microbial metabolites during Long Covid

Long COVID:

- Persistent immune activation
- Shift in tryptophan metabolism away from microbiota-derived protective indoles toward host-derived kynurenine pathway metabolites (potent AhR ligands).
- Chronic, maladaptive AhR activation,
- Immune exhaustion, epithelial dysfunction, neuroinflammation, dysautonomia, and impaired tissue repair

During acute Covid infection:
↑ IFN-γ, IL-6, TNF
↑ IDO1 expression (indoleamine 2,3-dioxygenase)

Result:
Tryptophan is shunted away from gut microbes
↓ fecal indoles (IPA, IAA), ↑ skatole / toxic metabolites

↑ kynurenine, quinolinic acid
This is adaptive short-term (limits viral replication).

Building up Long Covid:
The AhR switch flips — and doesn't flip back

Kynurenine + inflammatory indoles → persistent AhR Activation

Different ligand → different transcriptional program

This is the key pathology:
AhR is activated continuously, but with the wrong ligands.

Symptoms Long-Covid/Chronic AhR signaling

Long-Covid = a failure of immune resolution driven by chronic mis-activation of environmental sensing pathways (especially AhR).

Immune System
- T-cell exhaustion
- Impaired memory formation
- Blunted antiviral responses
- Paradoxical inflammation + immunosuppression

→ **explains reinfections, poor vaccine responses in some patients**

Brain & Nervous system

AhR is active in microglia, astrocytes and endothelial cells

Effects:
- neuroinflammation
- altered serotonin / dopamine synthesis (tryptophan depletion)
- autonomic instability

→ **brain fog, fatigue, POTS-like symptoms**

Gut & epithelial barriers
- ↓ tight junction integrity
- ↓ regenerative signaling
- Dysbiosis reinforces itself (loss of indole producers)

→ **"leaky gut" feeds systemic inflammation**

Mitochondria & Metabolism

AhR interferes with:
- mitochondrial biogenesis
- NAD^+ balance
- oxidative phosphorylation

→ **post-exertional malaise, chronic fatigue exercise intolerance (PEM)**

Most consistent stool metabolites signature

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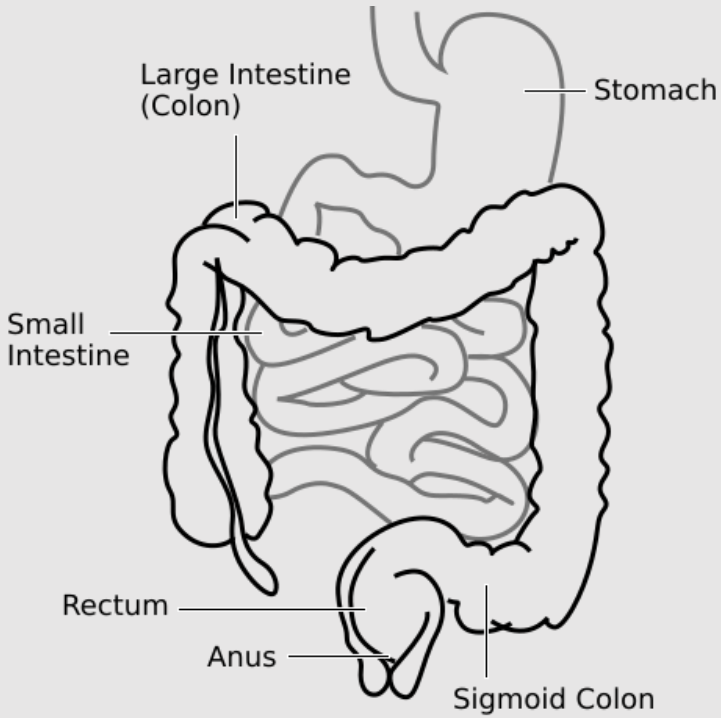
Downregulated ecosystem outputs

- ↓ SCFAs (butyrate, propionate, acetate, valerate)
- ↓ Secondary bile acids (e.g., DCA, LCA)
- ↓ Protective indole derivatives (e.g., IPA in some cohorts)

Upregulated signals linked to proteolytic fermentation

- ↑ Phenolics (p-cresol → p-cresol sulfate)
- ↑ Indole flux favoring indoxyl sulfate (host–microbial co-metabolite)



Interpretation

A shift from carbohydrate fermentation → protein fermentation, alongside loss of barrier-supporting metabolites.

Metabolome and the **Probiotic therapy**

Metabolites measured by Biovis – potential to modulate with Microbiome Center strains

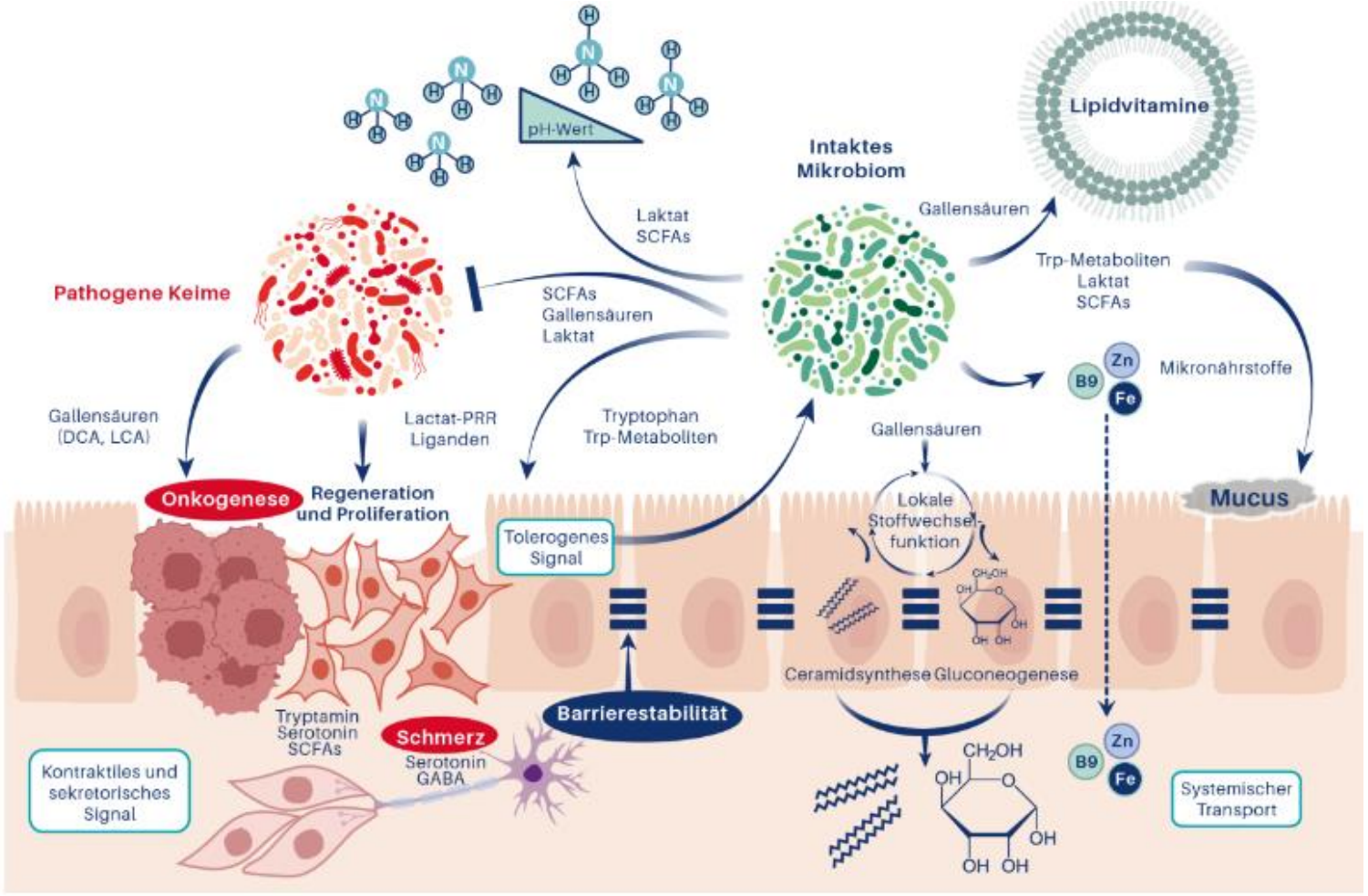
1. Tryptophan Metabolism
- Serotonin pathway – Serotonin
- Kynurenine pathway – Kynurenine
- Indole pathway (AhR signaling) - indole, IPA, IAA, ILA

2. Tyrosine Metabolism
- Tyrosine

3. GABA

4. Proteolytic Toxins
- Tryptamine
- p-cresol sulfate
- Indoxyl sulfate

5. Histamine
6. Bile acid metabolism
7. Phenylalanine Metabolism



Approach: the same philosophy as explained in our recent publication

BRILL WAGENINGEN ACADEMIC

BENEFICIAL MICROBES (2026) DOI:10.1163/18762891-BJA00110

Beneficial Microbes

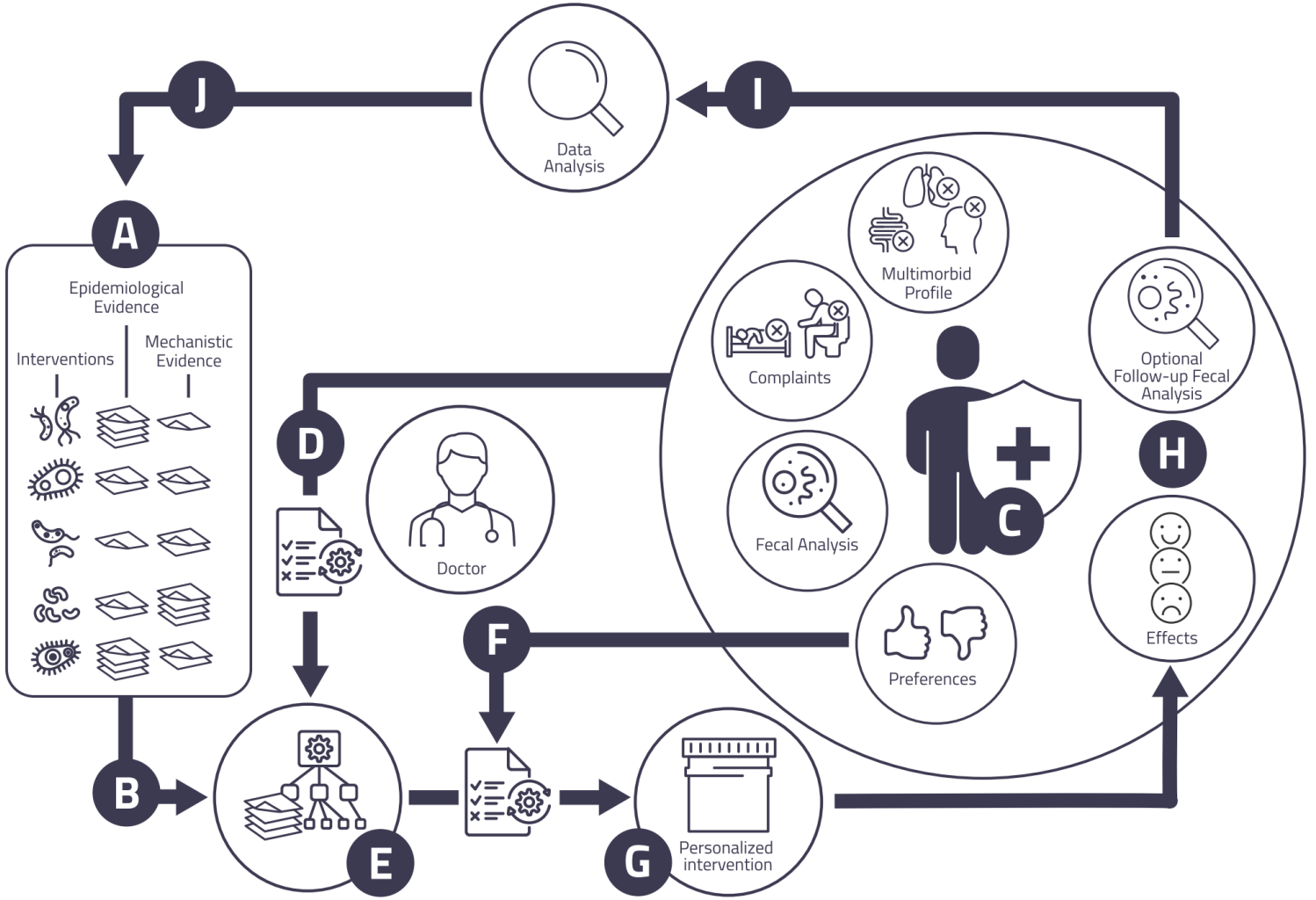
brill.com/bm

REVIEW ARTICLE

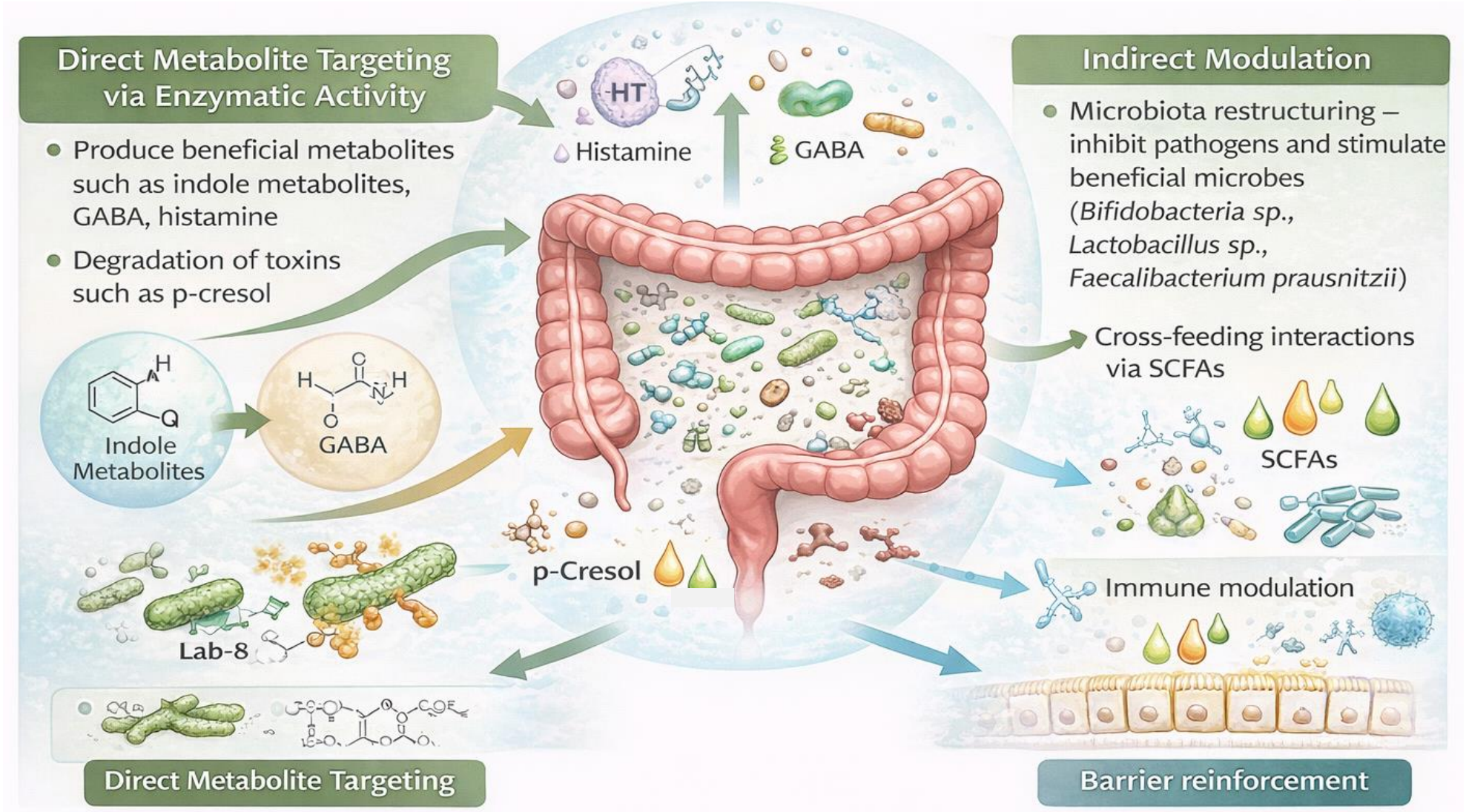
A pragmatic approach to integrate evidence-based medicine and personalized medicine: the example of personalized microbiome-targeting interventions

D. Zeilstra[1,2], A.A. te Velde[3], G. Remmers[4], I. Besseling-van der Vaart[5], R.J. Brummer[6] and A.D. Kraneveld[7]*

[1]Nutriz, Deventer, the Netherlands; [2]Microbiome Center, Zoetermeer, the Netherlands; [3]Amsterdam UMC



How probiotics influence metabolic pathways



Metabolic modulation – based on initial strain screenings

Tryptophane Metabolism

Increased tryptophane amino acid availability

Tryptophan → Serotonin
- Shift toward serotonin synthesis

Tryptophan → Kynurenine
- Shifts tryptophan away from kynurenine pathway

Tryptophan → Indole
- Microbiota stabilization

Indirect support of aromatic amino acid balance

Tyrosine Metabolism

Tryrosine/DOPA
- ↑ tyrosine and DOPA levels
- increase precursor availability

Dopamine pathways
- ↓ TH and DBH expression
- stabilization of dopamine/norepinephrine stress axis

Toxins

- Not direct measurements on the metabolism of p-cresol sulfate and indoxyl sulfate
- Reduction of pathogenic taxa and shift toward saccharolytic fermentation.
- ↓ pathogenic load
- ↑ Faecalibacterium and Bifidobacterium → lower toxin production

Histamine/ GABA

- Reported GABA stimulation
- Supports metabolic balance

Tryptophane Metabolism

Microbiome Center

L. plantarum DR-7

Strong evidence for animal and human studies[1,2,3,4]

Tryptophan → Serotonin
- ↓ IDO / ↓ TDO
- ↑ TPH2/TH2
- ↑ 5-HT6 receptor expression

Suggests shift toward serotonin synthesis

Tryptophan → Kynurenine

↓ IDO & TDO expression

Suggests shifts tryptophan away from kynurenine pathway

Increased tryptophane amino acid availability

→ Rebalancing towards a serotonin-favoring metabolic phenotype
→ Reduced inflammatory diversion

L. paracasei Lpc-37

Some evidence for human study (symbiotic)[5] **and fermentation model**[6]

Tryptophane availability
- ↑ Plasma tryptophan
- Increased conversion into bioactive derivatives in fermented matrix

Tryptophan → Serotonin
- ↓ 5-H significantly reduced in plasma

Suggest normalization of peripheral serotonin levels

Tryptophan → Kynurenine
- ↑ Kynurenine, ↓ 5-HT, ↓ 5-HIAA Suggests redistribution away from hyper serotonergic state

Tryptophan → Indole
- Indolelactic acid enriched in fermentation model

→ Redistribution away from hyper serotonergic state
→ Rebalancing of tryptophan metabolism

E. coli MC231

Good evidence for animal studies

Tryptophane availability
- Modulation of tryptophan utilization

Tryptophan → Serotonin
- ↑ 5-HTP
- Suggests shift toward serotonin synthesis and serotonin bioavailability in gut tissues[8]

Tryptophan → Indole
- ↑ Indole generation from tryptophan[7,9]
- Suggest increase of indole and indole derivates

→ Rebalancing toward a serotonin-favoring metabolic phenotype
→Rebalancing towards indole metabolism

L. plantarum P-8

B. adolescentis SH001

1.Zaydi et al., *Microbes* 2020; **11**: 753–66
2.Yap eta al., *Appl Biochem Biotechnol* 2020; **191**: 226–44.
3.Liu et al Adults. *Int J Mol Sci* 2020; **21**: E4608.
4. Chong et al., *Benef Microbes* 2019; **10**: 355–73.
5. Wang et al. *Research* 2020; **157**: 104784.
6. Loh et al., *J Agric Food Chem* 2021; **69**: 14024–36.
7. Vaaben, T. H. et al.EMBO Rep.26,.1688–1708.(2025)
8. Nzakizwanayo, J. *et al.Sci Rep*.5,.17324.(2015)
9. Dimopoulou, C. *et al.FEMS Microbiology Letters*.370,.(2023)

Tyrosine Metabolism

L. plantarum DR-7	*L. paracasei* Lpc-37	*E. coli* MC231	*L. plantarum* P-8	*B. adolescentis SH001*
Tryrosine/DOPA[1,2] ↑ tyrosine and DOPA levels increase precursor availability **Dopamine pathways** ↓ TH and DBH expression stabilization of dopamine/norepinephrine stress axis	**Tryrosine** [3,4] ↑ **L-tyrosine in plasma** Indicates aromatic amino acid modulation **Dopamine turnover markers** ↑ Homovanillic acid and ↑ vanillylmandelic acid •Suggests improved catecholamine metabolism regulation			
→ Stabilization of dopamine/norepinephrine stress axis → Reduced stress-associated metabolic overdrive	→ Improved catecholamine metabolism regulation → Possibly improved stress adaptation			

1.Zaydi et al., *Microbes* 2020; **11**: 753–66
2.Yap eta al., *Appl Biochem Biotechnol* 2020; **191**: 226–44.
3.Wang et al. *Research* 2020; **157**: 104784.
4. Loh et al., *J Agric Food Chem* 2021; **69**: 14024–36.

GABA

L. plantarum DR-7	*L. paracasei* Lpc-37	*E. coli* MC231	*L. plantarum* P-8	*B. adolescentis SH001*
	GABA Modulate GABA receptors, not GABA itself[3]. Suggests modulation of central inhibitory neurotransmission and stress response regulation	**C12AsnGABA*OH*** •Produces C12AsnGABA*OH*[4] •This GABA containing compound inhibits neuronal activation via the $GABA_B$ receptor	**GABA** •↑ GABA in clinical study[1]. •May be linked to ↑ neuroactive taxa (*B. adolesentis*); improved microbial metabolic pathways predicted by metagenomics	**GABA** •↑ GABA production[2] •Glutamate decarboxylation activity or stimulation of GABA-producing consortia **Folate** •De novo folate biosynthesis
	→ indirect GABA Neuro-Metabolic Support	→ analgesic effect via $GABA_B$ receptor	→ GABA Neuro-Metabolic Support	→ GABA & Folate-Driven Neuro-Metabolic Support

1.Ma et al., 2020 DOI:10.1007/s00394-020-02437-4
2.Producer leaflet
3,Stenman et al., *Behavioural Brain Research* 2020; **379**: 112376
4. Pérez-Berezo, T. *et al.Nat Commun*.8,.1314.(2017)

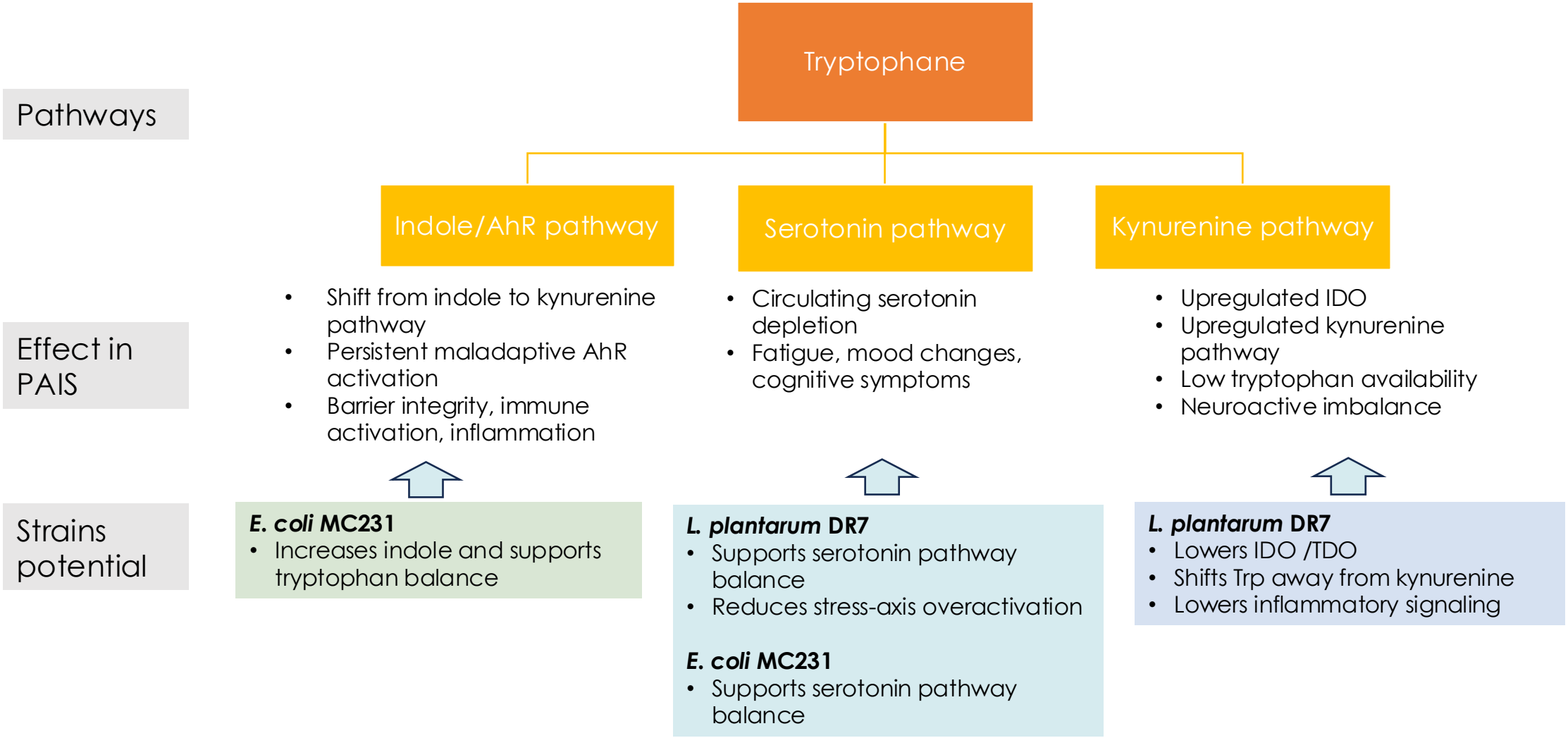
Proteolytic Toxins – p-cresol sulfate, tryptamine and indoxyl sulfate

L. plantarum DR-7	*L. paracasei* Lpc-37	*E. coli* MC231	*L. plantarum* P-8	*B. adolescentis SH001*
	•No direct measurements on the metabolism of p-cresol sulfate and indoxyl sulfate •Reduction of pathogenic taxa and shift toward saccharolytic fermentation. •↓ pathogenic load •↑ Faecalibacterium and Bifidobacterium → lower toxin production [1,2]	•No direct measurements on the metabolism of p-cresol sulfate and indoxyl sulfate ↓ Inhibit several bacterial pathogens, including *Pseudomonas aeruginosa, E. coli, Klebsiella pneumoniae, Enterococcus faecalis, Enterobacter cloacae*	•No direct measurements on the metabolism of p-cresol sulfate and indoxyl sulfate •↓Enterobacteriaceae/ •Proteobacteria; shift from proteolytic to saccharolytic fermentation - Reduced systemic toxin burden, improved epithelial and metabolic health [3,4,5]	No direct measurements on the metabolism of p-cresol sulfate and indoxyl sulfate •Shift from proteolytic to saccharolytic fermentation - potential ↓ toxin generation •Crossfeeder of F. prausnitzii, the major butyrate prodcuer

1. Ryan et al 2021 Integr Med (Encinitas) 2021
2. Hemalatha 2017 Microb Ecol Health Dis 2017; 28: 1298340
3. Kwok et al 2015 *Benef Microbes* 2015; **6**: 405–13.
4. Wang et al 2014 *Nutrition* 2014; **30**: 776-783.e1
5. Xu et al., 2020 DOI:10.1007/s00394-020-02437-4

Long COVID: Metabolic Imbalance & Probiotic Modulation

Microbiome Center



Long COVID: Metabolic Imbalance & Probiotic Modulation

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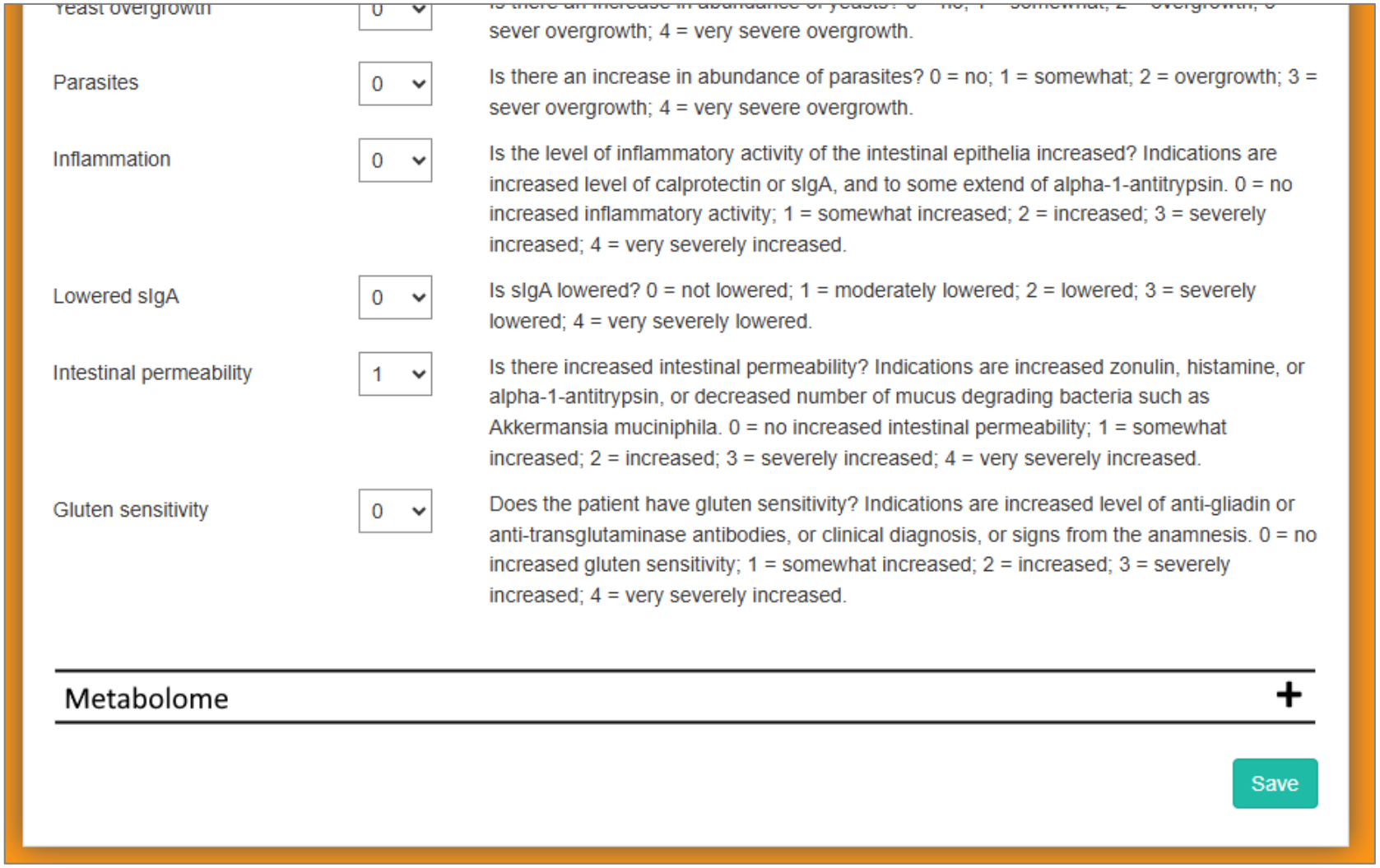
Pathways	Microbiota/ SCFAs	Tyrosine	GABA
Effect in PAIS	• Lowered butyrate producers • Increased proteolytic metabolism • Barrier weakening	• Lowered tyrosine and elevated phenylalanine/tyrosine • Autonomic dysregulation • Stress-axis imbalance	• Neuroinflammatory states often show imbalance of GABA • Some Long COVID patients show lowered GABA in the brain
Strains potential	***L. plantarum* P8** • ↑ Lactobacillus, Bifidobacterium, Faecalibacterium • ↓ Proteolytic fermentation↓ p-cresol, indoxyl sulfate ***L. plantarum* DR7** • ↑ SCFA-producing taxa (e.g., Blautia) • Supports fermentative balance ***B. adolescentis* SH001** • Crossfeeder of F. prausnitzii, the major butyrate prodcuer ***E. coli* MC231** • Strong inhibitor of proteolytic pathogens	***L. plantarum* DR7** • Stabilizes dopamine/norepinephrine pathways • ↓ Stress-associated enzyme expression • ↓ Cortisol ***L. paracasei* Lpc-37** • ↑ Tyrosine metabolism modulation • ↑ Dopamine turnover markers (HVA, VMA) • Balanced catecholamine processing	***L. plantarum* P8** • Supports neuroactive taxa that produce GABA • May act via interaction with *B. adolesentis* • Indirect gut–brain modulation ***B. adolescentis* SH001** • Produces GABA ***E. coli* MC231** • Produces compound that is agonist for GABAB receptor, with analgesic effects.

Implementations at MC platform



The metabolome input will be a separate, open-clickable section, so that it doesn't distract you when you did not measure these parameters

Summary:

- PAIS has high prevalence, is debilitating condition
- Clear link with microbiome dysbiosis
- Metabolome deviations found, links directly to symptoms
- Metabolome can inform clinical decision making/treatment:
  - Specific probiotics/ingredients can target metabolic pathways
  - Useable for clinical follow-up

- Microbiome Center will implement the metabolome in the forthcoming weeks

Thank you for your attention!