Setting The Clinical Scene

Michael Ash DO, ND, BSc, RNT
The Kiss by Gustav Klimt

Love, Glamour Romance

‘Immunity in equilibrium’
Tense,
scratching back,
face turned away,
he is all over her,
not a mutual kiss,
equilibrium is easily
misrepresented and lost.

“A Fatal Embrace”
Bugging inflammation: role of the gut microbiota


The Inverse Relationship Between Incidences of Infectious and Inflammatory Diseases

……..drastic changes to diet, with increases in consumption of carbohydrates and fats in highly processed foods, and decreases in the intake of dietary fibre. This forms the signature ‘Western diet', and translates to the average person living in a developed country ingesting about half of the 30 g of daily recommended intake of fibre. Both decreases in early-life microbe exposure owing to increased hygiene, and decreases in dietary fibre parallel major increases in the incidence of inflammatory diseases
The use of microbiota modulation to improve health is becoming a powerful strategy of inflammatory disease therapy, and microbiota intervention strategies can be designed using several approaches, including prebiotics, probiotics, postbiotics and FMT.
Gestation → Childhood → Senescence

Gestation:
- Vaginal microbiota modulation by prebiotic and probiotic
- Baby skin microbiota modulation by maternal vaginal fluids
- Placenta and oral microbiota modulation by orally administration of pre and probiotic

Childhood:
- Toddler Gut microbiota modulation
  - Healthy dietary habits, prebiotic and probiotic
- Breast-feeding
  - Breast milk modulation by administration of oral probiotic to pregnant or nursing mother

Senescence:
- Adult and elderly individual gut modulation
- Healthy dietary habits, prebiotic and probiotic
- Postbiotic administration
- Fecal microbiota transplantation
In developing our understanding of how dietary components shape the overall panorama of the gut microbiome, and the subsequent metabolite profile, we can identify the likelihood of events leading to inflammation and autoimmunity.
Emerging dietary treatments are not only economical, but also offer a non-invasive approach alternate to the risks of surgical procedures for chronic states of inflammation. Although it is in its early days, the implementation of diet and/or microbial metabolites or engineering the gut microbiota as a tool to prevent or treat inflammatory diseases is an exciting prospect that may have a great impact on human health.
Humans and microbes have established a symbiotic association over time, and perturbations in this association have been linked to several immune-mediated inflammatory diseases (IMID) including inflammatory bowel disease, rheumatoid arthritis, and multiple sclerosis.
IMID is a term used to describe a group of chronic, highly disabling diseases that affect different organ systems. Though a cornerstone commonality between IMID is the idiopathic nature of disease, a considerable portion of their pathobiology overlaps including epidemiological co-occurrence, genetic susceptibility loci and environmental risk factors.
The reduction of particular commensal microbes and a concomitant loss of their protective function possibly have a substantial impact on the course of disease. The ability of commensal bacteria to produce SCFA including butyrate, acetate and propionate via dietary fibre (a prebiotic) fermentation is a key benefit to the human gut.
As virome profiling in health and disease is a relatively new field, data are currently limited to investigations of IBD. With numerous viral infections as potential environmental triggers in IMID, particularly Epstein-Barr virus or cytomegalovirus in MS and several other lines of evidence that indicates a role for viruses, we anticipate future studies will target the gut virome in other IMID.
These include short-chain and medium-chain fatty acids (SCFAs and MCFAs, respectively) produced by fermentation of dietary fibres and carbohydrates; secondary bile acids converted from primary bile acids; metabolites generated from meat-derived choline and l-carnitine; and other lipids including conjugated fatty acids and cholesterol. Indole derivatives (for example, γ-aminobutyric acid), which affect the levels of brain-derived neurotropic factor in the central nervous system.
Our growing fundamental knowledge about how diet affects the composition of microbial communities and enzymatic conversion of by-products is changing our understanding of the symbiotic relationship between the microorganisms that populate our body cavities and the biologic responses required for host adaptation.

The Metabolic, Immunological Interface
Gut microbiota is considered as a major regulator of metabolic disease. This reconciles the notion of metabolic inflammation and the epidemic development of the disease. In addition to evidence showing that a specific gut microbiota characterizes patients with obesity, type 2 diabetes, and hepatic steatosis,...
the mechanisms causal to the disease could be related to the translocation of microbiota from the gut to the tissues, inducing inflammation. The mechanisms regulating such a process are based on the crosstalk between the gut microbiota and the host immune system.
The serum metabolome of insulin-resistant individuals is characterized by increased levels of branched-chain amino acids (BCAAs), which correlate with a gut microbiome that has an enriched biosynthetic potential for BCAAs and is deprived of genes encoding bacterial inward transporters for these amino acids.
Prevotella copri and Bacteroides vulgatus are identified as the main species driving the association between biosynthesis of BCAAs and insulin resistance, and in mice we demonstrate that P. copri can induce insulin resistance, aggravate glucose intolerance and augment circulating levels of BCAAs. Our findings suggest that microbial targets may have the potential to diminish insulin resistance and reduce the incidence of common metabolic and cardiovascular disorders.
Subjects who consume a Mediterranean diet rich in fruit, legumes and vegetables have higher levels of faecal short-chain fatty acids, regardless of the diet type.

Microbiota modulation through consumption of diets rich in diverse vegetable foods offers the prospect of increasing health and mitigating disease risk.

...propose a model of immunity that is based on equilibrium, in which the healthy immune system is always active and in a state of dynamic equilibrium between antagonistic types of response. **This equilibrium is regulated both by the internal milieu and by the microbial environment.** As a result, alteration of the internal milieu or microbial environment leads to immune disequilibrium, which determines tolerance, protective immunity and inflammatory pathology.
The equilibrium model of immunity.
The equilibrium model of immunity.

Mounting evidence indicates that nutrients and lifestyle strongly influence genome–metabolic functional interactions determining disease via altered epigenetic regulation. The mitochondrial network is a central player of the metabolic–epigenome–genome axis, regulating the level of key metabolites [NAD+, AcCoA (acetyl CoA), ATP] acting as substrates/cofactors for acetyl transferases, kinases (e.g. protein kinase A) and deacetylases (e.g. sirtuins, SIRTs).
Increasing evidence shows that the health status of the mitochondrial network in mammalian tissues is highly sensitive to the **cellular energy/redox capacity**, fusion–fission dynamics and mitochondrial turnover as a result of the dynamic balance between mitochondrial loss compared with scavenging and replacement, e.g. by mitophagy and biogenesis. Alterations of mitochondrial quality control due to altered fusion–fission dynamism and mitophagy can explain differences in cumulative mitochondrial damage.
The response of these processes to physiological conditions imposed by, e.g. diet, exercise, via the epigenome exert a direct impact on mitochondrial health and healthspan. Under disease conditions mitochondria can become dysfunctional exhibiting in general (at least) three main impairments: uncoupling of oxidative phosphorylation (OxPhos), excess ROS emission and abnormal Ca\(^{2+}\) uptake. These defects impair function by altering energy availability, the cellular redox environment and cell viability.
…..recent exciting advances surveyed herein show that this knowledge is underlain by a major and complex cell biology phenomenon dubbed the **mitochondrial quality control axis**, comprising mitochondrial turnover through biogenesis, fusion–fission dynamism and mitophagy. Being a convergent path of substrate degradation, recycling and signalling, mitochondrial healthy function, as revealed by aerobic capacity, represents a critical probe and index of health- and life-span.
The fluxome and the metabolic–epigenetic–genomic axis

fluxome, orange arrows; gene expression, blue arrows.

• Barrier Management
• Innate Detoxification
• The Interplay between Mitochondria, Microbiome and related Metabolomics in human health a practical target for health management?
• Antioxidant, Detoxification and Immune Support
• Gut Brain Axis analysis
• Clinical Application via case examples
The End
Many thanks for your attention
Understanding Gastrointestinal Permeability
Consequences and Interventions

David Quig, PhD
“To be or not to be a pathogen... that is the mucosally relevant question”
Phillippe Sansonetti (2011)
The Gastrointestinal (GI) Ecosystem

- **Metabolome** - Microbes *plus* their collective metabolites
- **Terrain** - Intestinal barriers
- **Gastrointestinal Glycobiome (Glycomics)**
  - Mucins - highly glycosylated proteins, constantly renewed
    - Primary molecules in host-pathogen interaction
    - Secretory and adherent - critical protective barrier layers
    - Dynamic and responsive to changing conditions
    - During infectious invasion the *distinct* mucin glycans of *both* the host and the pathogen change, which can affect pathogenesis and the host’s structural and immune response.

To be Covered...

• **Intestinal barriers**
  – the primary components
  – key regulatory factors (under appreciated Clostridium)
  – *indirect* assessment of key barrier components from CSA (bacteria, butyrate, sIgA, inflammation)
  – *direct* assessment of serum zonulin levels
  – therapeutic support for barrier integrity

• **Intestinal permeability** - transcellular and paracellular
  – delineation of zonulin pathway
  – consequences of sustained epithelial barrier dysfunction
Intestinal Permeability

Breaches in barriers are associated with:

- Gut-derived lipopolysaccharides and protein fragments
- Direct adhesion of bacteria to endothelial cells
- Changes in the microbiota / metabolome
  Infection, lifestyle and dietary factors (e.g. alcohol)
- Stress
- Pharmaceuticals (e.g. antibiotics, prednisone, NSAIDs)
- Environmental xenobiotics
- Inflammation / epithelial damage
- Cancer treatments
- Excessive exercise with heat stress

BMC Gastroenterol (2014)14:189

Scientific Rep(2014)4:5551 DOI: 10.1038/srep05551
Intestinal Barrier System

• Multi-factorial, layered and a highly integrated gradient
  
High maintenance ~ 40% of daily energy expenditure (BMR)

• Primary functions
  – \( \text{H}_2\text{O/electrolyte balance, and prevent the influx of microbes, toxicants, and antigens from the lumen of the gut} \)
  – Regulates \textit{appropriate} inflammatory/immune responses (tolerance or immune response)

• “Two way street”- mutual regulation of microbes and barriers
  – Commensal bacteria facilitate optimal status of the barrier components.
  – Barrier components provide \textit{surveillance, protection and selection} of commensal bacteria, and elimination of pathogens.


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Intestinal Barrier- *Multilayered System*

1. **Commensal bacteria** and their *metabolites*
2. **Functional chemical / biochemical barrier**
   Digestive secretions, immune molecules (*slgA*, antimicrobial peptides), and inflammatory mediators (cytokines)
3. “External” physical barrier
   Epithelial cell lining / tight junctions, mucus gradient, glycocalyx, lamina propria, vascular endothelium

   **“Leaky gut” = Epithelial Barrier Permeability**


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Microbiology - Insufficiency Dysbiosis

22 yof, broad-spectrum antibiotics. No GI symptoms, but developed arthritic-like pain/inflammation in her hand/fingers.

Compromised Intestinal Barriers

- Loss of key commensals
- ↓ butyrate production
- ↓ microbial-host cross talk
- ↓ sIgA
- ↓ antimicrobial peptides
- ↓ mucus / mucin secretion
- ↑ mucus and mucosal permeability

permeability and inflammation as well as systemic problems

Mucus and Mucosal Barriers - The Terrain


UC patients disrupted microbiome, ↓ mucus, ↑ pathogen colonization and permeability
Gut(2014)63:281-91

Proteases

ROS, proinflammatory cytokines

Mucus "blanket"

Gut lumen

Mucosa

Glycocalyx

membrane tethered mucins

Epithelium

Tight Junction

Endosome

Dendritic cells, T-cells, B-cells, lymphocytes

Mucosal Barriers

Pathogens

sIgA, antimicrobial peptides, ROS

Lamina propria

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Mucosal Cells- Sense, Report, Secrete

- Finely tuned network of immune mechanisms for microbial recognition; selection and eradication


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BMC Gastroenterol (2014)14:189
Mucins Maintain Gut Barrier Function

• Highly glycosylated proteins that form a polymeric gel-like network
  - Secretory (MUC2) - mucus barrier, habitat and nourishment
  - Cell bound (glycocalyx) - binding sites, barrier, signaling
  - Prevent direct binding to endothelial cells... “decoys”
  - Regulated by commensal bacteria within the mucosa (vs. lumen)
  - Mucin “harvesting” → glycans → ↑ butyrate → ↑ mucin production
  - Mucins are to endothelial cells as biofilm is to bacteria and yeast

• Obesity and high-fat (saturated) diets ↓ mucus thickness, and increase inflammation and permeability.

• Prebiotics (oligosaccharides) increase mucin-stimulating bacteria
  - Onions, garlic, asparagus, leeks, yams, chicory root, agave, bananas, artichoke (root type)

Recap - The Microbial/Host Mucin Cycle

**The Host’s Pre-biotic**

- The protective mucus layer at the epithelial interface is *continuously* reshaped/refreshed (about 5 L/day).
- It’s glycoproteins *harbor* and *feed* symbionts.
- A *consortium* of mucin-degrading specialists *within* the inner mucus layer release glycans that are fermented by other commensal bacteria that crank out *butyrate*.
- The butyrate “cross talk” stimulates mucin production and regulates immune function and inflammation... *fortifies the mucosal barrier.*
Clostridium Species Have a Key Regulatory Role

- Only 5 out of about 105 Clostridium species are true pathogens
- ≈ 5% of the bacteria in a **strategic position** *(glycocalyx)* that mediate continuous cross-talk with the mucosa *(major butyrate producers)*
  - Including *C. hystolyticum*, *C. lituseburensense*, *Clostridium cluster XIVa*
  - Integral symbionts in the regulation of barrier homeostasis
- ↓ abundance of *Clostridium spp.* with colorectal cancer and IBD
- ↑ abundance of Clostridium species is associated with consumption of high fiber intake from fruit, vegetables, and beans
- **Colonization in breastfed infants during the 1st month of life**


Vertical Transfer of Clostridium from Breastmilk to Infants

- **Human study (n=7)** - microbial analysis of breastmilk, and maternal and infant feces (16S rRNA pyrosequencing)

- In addition to the majority genera *Bifidobacterium*, commensal Clostridium species (*C. blautia, C. collinsella, and C. veillonella*)

- **Mechanism**: sub-endothelial dendritic cell "feet" sample and transport gut bacteria to lymph

www.nature.com/nrmicro/journal/v10/n1/fig.html
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Short Chain Fatty Acids (SCFA)

• Fermentation of *mucin-derived glycans* and *soluble dietary fiber* by commensal bacteria (*butyrate*, acetate, propionate)

• **Fuel** for enterocytes/many tissues, and maintenance of mucin and **barrier function**

• **Butyrate mediates microbial-host crosstalk**—“the host listens” to butyrate
  – Immune and epithelial cells through **G-protein-coupled receptors**
  – Mediates **production of mucins, antimicrobial peptides**, the release of **anti-inflammatory cytokines** by epithelial cells
  – Regulates expression of tight junction proteins
  – Systemic uptake—crosses blood brain barrier (can ↓ neuroinflammation)

• *Fecalibacterium prausnitzii* and some *Clostridia* clusters are major butyrate producers (**strategic real estate**)
  – More abundant in diets containing chick peas and raffinose

sIgA - The **BRICK** in the Mucus Barrier

- Anchored in the viscous mucus, and *on epithelial cells*
  - **Immune exclusion** of microbes (including viruses) and toxins
  - Binding to pathogens - ↓ diffusion, ↓ ATP production → loss of motility and ↓ *biofilm production*
  - **Anti-inflammatory** - Neutralizes LPS in apical recycling endosomes (abrogates NF-κB signaling)
  - **Antiparasitic augmentation** - *activation* of eosinophils
  - **Regulates the composition of the microbiota** - provides constant *surveillance/sampling* and *communication with immune cells*

- sIgA from maternal milk *initiates* immune training (vaginal birth), and epithelial maturation.

- Chronic Candidiasis is often associated with *low fecal* sIgA despite normal serum IgA levels *(sIgA specific protease enzymes)*


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Intervention for Low sIgA

- **Ω-3 and short chain fatty acids, olive oil, Zn, D, A**
- **Probiotics** (must see the strains)
  - *Lactobacillus rhamnosus GG* and *Bifidobacterium lactis* Bb-12
    (↑ number of sIgA secreting cells in formula-fed infants)
  - *S. boulardii* - ~75% ↑ sIgA (germ-free mouse model)
- **Prebiotics** vegetables, fruits, chickpeas, beans, etc.
- Glutamine
- Arginine (NO)
- **Stress decreases sIgA**

  Consider evaluation of salivary cortisol and DHEA
  
Vitamin D and GI Restoration/Health

- Vitamin D receptor (VDR) signaling turns off chronically activated T cells
  - Decreases mucosal inflammation
  - ↓ T cell proliferation / inflammatory cytokine production
  - Enhances expression of EC tight junction proteins
- Influences the microbiome and barrier integrity by regulation of antimicrobial peptides
- Facilitates mucosal barrier homeostasis and colonic epithelial healing

Am J Physiol Gastrointest Liver Physiol(2008)294:G208-16

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Breaching the Epithelial Barrier

Transcellular
- Electrolytes, B-12, AAs, sugars, SCFA, sIgA, [antigens, BT]
- Receptors/endocytosis

Paracellular
- Passive diffusion
- Water, solutes

Commensals
Pathogens, toxins, antigens
sIgA

↑ BT

Overt immune response

Tolerance

©Doctors Data, Inc.
Clin Nutr(2015)34:1080-87 (excellent review)
The Cellular Barrier

Zonulin-induced Permeability of the Epithelium

• Tight junctions are *dynamic, interconnected protein networks* that regulate paracellular influx for all epithelial cells (*GI, heart, brain*)

• Zonulin is currently the only known *physiological reversible modulator* of intercellular tight junctions (TJ)

• *Transient reversible* opening- “influx of bacteria facilitates a defense mechanism- bacterial flush with a robust immune response “ *

• *Sustained* high serum zonulin levels occur with *autoimmune diseases;* Celiac, Crohn’s, RA,T1D; *cancers;* gliomas, breast, ovarian, pancreatic, *neurological;* demyelinating polyneuropathy, MS, schizophrenia, and *juvenile fatty liver disease, asthma, and metabolic syndrome*
Gliadin-induced Zonulin Release, Increased Intestinal Permeability, and Onset of Autoimmunity

Gliadin Peptides → cytokines, inflammatory and immune response → tissue damage

CXCR3 and zonulin are over expressed with CD
Gluten Sensitivity- Not Just a Fad

• When people with GI diseases such as Celiac disease, NCGS or IBS consume gluten proteins they can become “sick.”

Photo from unsplash.com

©Doctors Data, Inc.
Increased Zonulin - Not Just Celiac

Barbaro MR, Volta U et al. UEG Week 2014, DDW 2015
Serum Zonulin and Increased Permeability Respond to a Gluten-free Diet

- Zonulin (serum antigen) levels assessed in pts. with Celiac disease, NCGS, IBS and IBDs- on and off gluten

- Gluten-free diet (GFD)
  - IBDs  No significant effect of GFD on elevated serum zonulin levels
  - Celiac  ↓ zonulin and ↓ antibody titers for tTG and DGP
  - NCGS  ↓ zonulin and anti-gliadin antibodies
  - IBS-D  greater  ↓ zonulin in subjects with double HLA-DQ alleles

- A gluten-free diet can be beneficial to patients who have elevated levels of serum zonulin and experience reactions to gluten.


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## Zonulin as a Diagnostic Marker

<table>
<thead>
<tr>
<th></th>
<th>RESULT / UNIT</th>
<th>REFERENCE INTERVAL</th>
<th>PERCENTILE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Zonulin*</td>
<td>55 ng/mL</td>
<td>&lt;45</td>
<td></td>
</tr>
</tbody>
</table>

- ↑ serum zonulin (antigen) levels indicate increased permeability.
- Correlated with TEER, and a high urine lactulose : mannitol, but...
- The small size of lactulose (342) may preclude definitive information about the influx of macromolecules.

**“Triggers” for increased zonulin (permeability)**

Gliadin, *direct adherence of any* bacteria, stress, chemokines (inflammation), bacterial LPS, enterotoxins/proteases, corticosteroids, dietary protein fragments

[maybe food additives-microbial transglutaminase, fructose, emulsifiers, salt, nanoparticles — correlation data, some animal studies]*


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Blockade of the Zonulin Binding

**Proof of Concept**

- Increased permeability of the small bowel precedes the onset of diabetes and pancreatic islet destruction (humans years)*
  - BBDP rats – develop T1D within about 80 days
  - **Blockage of zonulin binding** to enterocytes by a synthetic peptide (AT1001) prevented zonulin-induced intestinal permeability, insulitis and **decreased the incidence of diabetes by 70%** (*despite markedly increased luminal zonulin*)

- **RDBPC trials (CD, n=342)** AT1001 was well tolerated, blocked IP, and greatly ameliorated GI symptoms and TNF-γ levels after a **14 day gluten challenge** (Larazotide™ tested in ~ 500 pts.)


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Clinical Intervention to Normalize EC Tight Junctions

- **Eliminate triggers** (including inflammation)
- **Support for expression** of tight junction proteins
  - Specific probiotics*
  - Prebiotics and butyrate
  - Glutamine, tryptophan
  - Curcumin
  - Vitamin D and retinol
  - Quercetin, genistein
  - γ-linoleic acid
  - Α β-casein peptide
- Chitosan and ethanol decrease expression of TJ proteins


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Take Away points

• The *supra* endothelial barrier is a complex ecosystem of resident symbionts, resident and recruited immune cells, and very responsive mucus layers.

• The 1\textsuperscript{st} line mucus barrier harbors *commensals* and *immune components* that support the barrier.

• EC membrane-bound mucins (glycocalyx) serve as adhesion sites, and protect and *communicate* with the epithelium.

• Mucus and it’s mucins are dynamic, and affected by symbionts and pathogens- clinical intervention can be effective and should be part of gut restoration protocols.

• Zonulin is a reversible physiological regulator of epithelial cell tight junctions (permeability of the epithelium).
Take Away Points (cont’d)

• Persistent activation of the zonulin pathway predisposes individuals to inflammatory, autoimmune and even neoplastic disorders... as well as NCGS.

• Elucidation of the zonulin pathway has facilitated development of a diagnostic marker of intestinal permeability to proinflammatory and antigenic macromolecules.

• Serum zonulin levels can be measured serially to monitor your clinical interventions to restore EC tight junctions.
The Interplay Between Mitochondria, Microbiome and Related Metabolomics in Human Health - a Practical Target For Health Management?

Michael Ash. DO, ND, BSc, RNT
Overview
Inflammation Bugs and NCDS
Key Receptors – How we translate food into medicine
SCFAs - *Your metabolically driven inflammation modeller.*
   *An evolving story of fibre, bacteria and geography*
GPCR’s: The Important Lock
SCFAs Metabolites and Nutrients
Breaking Bad ‘Mitochondria’
Barrier and Molecular Energetics
Bacteria as Immune Regulators
Nutrients as Targeted (SNP) Therapy
Nutrition and the immune system are highly inter-connected: nutrition and metabolism clearly impact the immune system and immune responses, in turn, influence metabolism.
Attendees should:

- Understand the intimate and interconnected relationship between organisms and us in the context of a rapidly changing digestive tract.
- Translate the consequences of dysfunction in multiple systems into clinical stories.
- Recognise value points for clinical intervention in the digestive tract.
- Be able to describe how we are consortia, in that most of our genes and many of our metabolites are microbial, and that the gut related metabolome is the point of data analysis for the future.
- Understand that the associated mucosal immune systems remain a highly useful point of safe predictive intervention.
“Identifying the best target for a heterogeneous collection of patients in whom diverse immunopathogenic mechanisms are activated requires multi-layered, iterative strategies to achieve safe effective outcomes.”
The microbiota and its metabolic machinery produce a myriad of metabolites that serve as important messengers between the diet, microbiota, and host. **Short-chain fatty acids affect immune responses and epithelial integrity via G-protein–coupled receptors and epigenetic mechanisms.** By increasing our understanding of interactions between diet, immunity, and the microbiota, we might develop food-based approaches to prevent or treat many diseases.
# Effects of Various Diets on Microbiota and Immunity

<table>
<thead>
<tr>
<th>Foods (food components)</th>
<th>Microbiota-dependent</th>
<th>Involved pathways</th>
<th>Effect on immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Inflammatory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Red meat, eggs, milk</td>
<td>+</td>
<td>TMA</td>
<td>Atherosclerosis †</td>
</tr>
<tr>
<td>(contain phosphatidylcholine, L-carnitine)</td>
<td></td>
<td>TMAO</td>
<td>(proinflammatory cytokines, forward cholesterol transport)</td>
</tr>
<tr>
<td>High-fat diet</td>
<td>+</td>
<td>Intestinal permeability ↑</td>
<td>Endotoxemia</td>
</tr>
<tr>
<td>Milk-derived fat</td>
<td>+</td>
<td>TLRs</td>
<td>Intestinal cytokine expression ↑</td>
</tr>
<tr>
<td>Salt</td>
<td>?</td>
<td></td>
<td>Intestinal and systemic inflammation↑</td>
</tr>
<tr>
<td><strong>Anti-inflammatory</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cruciferous vegetables</td>
<td>+</td>
<td>AhR ligands</td>
<td>IL22 †, maintenance of</td>
</tr>
<tr>
<td>(carbazoles)</td>
<td></td>
<td></td>
<td>intraepithelial lymphocytes and</td>
</tr>
<tr>
<td>Vegetables, fish</td>
<td>+</td>
<td>AhR ligands</td>
<td>IL22 †, mucosal protection from</td>
</tr>
<tr>
<td>(tryptophan)</td>
<td></td>
<td>GPCRs</td>
<td>inflammation</td>
</tr>
<tr>
<td>Soluble fibre</td>
<td>+</td>
<td>SCFA</td>
<td>Mucus production↑</td>
</tr>
<tr>
<td>(complex carbohydrates)</td>
<td></td>
<td></td>
<td>IgA production↑</td>
</tr>
<tr>
<td>Mediterranean diet</td>
<td>?</td>
<td>GPCRs (Gpr41, Gpr43, Gpr109a)</td>
<td>Proinflammatory cytokines ↓</td>
</tr>
<tr>
<td>(enriched in ω-3 fatty acids)</td>
<td></td>
<td>Gpr120</td>
<td>Tregs↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Proinflammatory cytokines ↓</td>
</tr>
</tbody>
</table>
Figure 3 Anti-inflammatory effects of food components. (A) AhR provides an important link between the intestinal immune system and food-derived ligands. AhR activating ligands include indolo(3,2-b) carbazole or 6-formylindolo(3,2-b)...
Key Nutrients Explored

250mg – 1000mg Saccharomyces Boulardii
1 billion – 30 billion Lactobacillus GG, Plantarum, Rhamnosus, Bifido Bifidus
(or other IL10 inducing probiotics)
Iron binding Probiotics

- Propionibacterium freudenreichii/ Escherichia coli Nissle 1917
Diet rich in Ahr ligand foods and SCFA promoting foods
12,000-50,000iu of Vitamin A (Retinol Palmitate)
2,000-50,000iu Vitamin D3
MitoQ 5-10mg daily (or ubiqinol ≥100mg) BID
Membrane Replacement Therapy – 1000mg-1500mg TID
B12 supplementation
Aloe Vera, Oral 50ml, Butyrate
Key Lifestyle Events Explored

Time Restricted Feeding (TRF)
Focused Exercise
Metabolic Integration with Mitochondria
Reduced Exogenous Exposure
Inhibition of Inflammasomes
Microbial Diversification
Metabolite Induction/Modification
xylooligosaccharides (XOS)
Barrier Management
Overview

2 Inflammation Bugs and NCDS

3 Key Receptors – How we translate food into medicine

4 SCFAs - *Your metabolically driven inflammation modeller.*
   An evolving story of fibre, bacteria and geography

5 GPCR’s: The Important Lock

6 SCFAs Metabolites and Nutrients

7 Breaking Bad ‘Mitochondria’

8 Barrier and Molecular Energetics

9 Bacteria as Immune Regulators

10 Nutrients as Targeted (SNP) Therapy
Rapid environmental transition and modern lifestyles are likely driving changes in the biodiversity of the human gut microbiota. With clear effects on physiologic, immunologic, and metabolic processes in human health, aberrations in the gut microbiome and intestinal homeostasis have the capacity for multisystem effects. Changes in microbial composition are implicated in the increasing propensity for a broad range of inflammatory diseases, such as allergic disease, asthma, inflammatory bowel disease (IBD), obesity, and associated noncommunicable diseases (NCDs).
“the only good bug is a dead bug”
Effects of Drugs on Bugs

A Longitudinal Analysis Of The Infant Gut Microbiome Reveals Decreased Microbial Diversity After Antibiotic Therapy.

Delivery mode (vaginal versus caesarean) also had strong long-term effects on microbial diversity.
"One of the key motivations of microbiome research is that the microbial population of early childhood appears to be critical to human health, in that decreased diversity of the gut microbiome has been implicated in a number of allergic and autoimmune diseases,"
Antibiotics are useful for treating susceptible bacterial infections and certainly provide human health benefits. However, with the emergence of multidrug-resistant pathogens that are predicted to kill 10 million people a year by 2050, there is a dark side to antibiotics. The mechanism described by Faber et al. sheds light on another dark side. Indeed, increasing numbers of studies now describe mechanisms used by gut pathogens to take advantage of the post-antibiotic period to hyper-replicate within the gut and successfully infect the host.
A more holistic understanding of what constitutes gut health will ultimately guide future approaches to correcting gut dysbiosis and the answer surely lies in the consideration of the entire microbial ecosystem rather than its individual components.

Dysbiosis

"a relationship of non-acute non-infectious host-microorganism interaction that adversely affects..."
The Knowledge SHIFT
..much of the research on infectious diseases continues to be dominated by reductionist approaches; one variable is altered while all others are assumed to hold constant.

Microbiologists tend to view the microbe as the key variable in disease and treat the host as a constant. Immunologists generally see the microbe as a constant and the host response as the variable.
This term should take in not just microbes, but the wider 'exposome' and recent discoveries in infection and immunity research.

A term is needed that encompasses sequences from the environment — intrinsic or extrinsic — that impart pathogenic or benign information to eukaryotic immune receptors.

‘PERCEPTOGEN’ (microbial or environmental) to cover protein sequences that affect the body's range of reactions after perception by its immune receptors.
Louis Pasteur 1861

Infectious medicine 1% of Bacteria cause human disease millions a year still die from them

Robert Koch Postulates 1875

> 75% Die from NCD

Tolerance & Health

Carl Bensussan 2011 Immune Maturation Inflammation

Pathobionts

Germ Theory

Commensals

1667 Antoni van Leeuwenhoek Motile microorganisms

Elie Metchnikoff 1908 LAB

Turnbaugh 2007 Metagenomics 16S rDNA

Probiotics

Prebiotics

Faecal Transplants

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Probiotics

Prebiotics

Faecal Transplants
The healthy IM cannot be defined as an absolute entity anymore, but it needs to be reconsidered as a dynamic factor, with its own degree of ability to change over time preserving the individual homeostasis through the human lifespan and in response to a changing environment.
Over the past 50 years, humans have changed ecosystems more rapidly and extensively than in any comparable period of time in human history, largely to meet rapidly growing demands for food, freshwater, timber, fibre, and fuel. This has resulted in a substantial and largely irreversible loss in the diversity of life on Earth.
Gut microbial community development is an example of ecological succession, starting when the embryonic intestinal organ is developing in the uterus. We can apply Waddington's notion of an epigenetic landscape, and consider the ecological dynamics of the gut microbiota in a similar way to the development of any other human organ presenting phenotypic changes from ontogeny until death.
The **microbiota** should be considered as just another component of the human epigenetic landscape. Thus, health is also a reflection of the diversity and composition of **gut microbiota** and its metabolic status.

"The Nation that destroys its soils destroys itself" – Franklin D Roosevelt
Deep sequencing of the gut microbiomes of 1135 participants from a Dutch population-based cohort shows relations between the microbiome and 126 exogenous and intrinsic host factors, including 31 intrinsic factors, 12 diseases, 19 drug groups, 4 smoking categories, and 60 dietary factors.
It is becoming increasingly apparent that our diet, gut microbiota and health are inextricably linked. We must be conscious that, when we make dietary interventions, we affect the growth of trillions of bacteria.
……depends on the ability of the microbiota to react and return to the pre-disturbed state, that is, one in which the microbiota is considered stable. This notion of Stability, however, must be considered from both the standpoint of Composition (Diversity), Redundancy and Function.
The concept of the mammalian ‘supraorganism’, with the gut microbiota collectively acting as a major virtual organ that augments host metabolism and physiology, has resulted in a paradigm shift in understanding human biology and medicine.

‘Metabotypes’ vary extensively between individuals and populations, and result from the complex interplay between host genes, lifestyle, diet and gut microbes.
Demonstrate that the gut microbiota can be used to predict individualised blood glucose responses to particular foods, which differ between individuals.
Bottom-up approaches emphasising microbiota-derived metabolites - Metabolomics data can complement data derived from standard taxonomic approaches (e.g. 16S sequencing).

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In molecular biology and genetics, a transcription factor is a protein that binds to specific DNA sequences, thereby controlling the rate of transcription of genetic information from DNA to messenger RNA.

Regulation of transcription is the most common form of gene control. The action of transcription factors allows for unique expression of each gene in different cell types and during development.
AhR – How can I help?

- the ligand-activated transcription factor aryl hydrocarbon receptor (AhR) has been studied for many years by toxicologists as the receptor mediating the toxic effects of the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin.
- However, AhR ligands are provided by the diet and are also generated in the gastrointestinal tract as a result of complex interactions between the host and the commensal flora.
Ahr has been shown to regulate different cell populations in the immune system including (transcription) RORγt⁺ group 3 innate lymphoid cells (ILCs), T helper (Th)17/22 cells, γδT cells, regulatory T cells (Tregs), Tr1 cells, and antigen presenting cells.

A better understanding of the function of Ahr in the gut is important for developing new therapeutic means to target Ahr in future treatment of infectious and autoimmune diseases. Ahr has important effects in the regulation of the immune response, making it a potential therapeutic target to control immune mediated disorders.
……highlight the evolutionarily highly conserved AhR system as a previously unknown link between external environmental stimuli and the maintenance of specialized immune cell populations (IELs), as well as the control of the microbiota.

Our results provide a **molecular basis for the importance of cruciferous vegetable-derived phytonutrients as part of a healthy diet in sustaining important elements of the immune system and in controlling bacterial colonization**. Furthermore, we reveal an important role for AhR, independent of xenobiotics, in the physiology and homeostasis of epithelial barrier sites.
Small and Large Intestine
Without HCL, Indole 3 ligands are not extracted.

- PPI?
- Betaine HCl?
- DIM, I3C?

In this study which evaluated the largest number of long-term PPI users reported to date, **we were able to detect significant differences in the beta-diversity as well as the relative abundance of bacterial taxa between PPI users and non-users.** Most notably, there was an increased ratio of Firmicutes to Bacteroidetes among PPI users, as well as significant differences in the abundance of other genera and species between long-term PPI users and PPI non-users.

Increasing risk of dysbiosis and pathogenic infection from CDI
A majority of natural AhR ligands are introduced into biological systems by oral consumption of foods and herbal medicines, as in the cases of flavonoids, stilbenes, carotenoids, and indoles. Indole compounds, such as indole-3-carbinol (I3C) and its chief metabolite 3,3’- diindolylmethane (DIM), are found in cruciferous vegetables, including broccoli, brussels sprouts, cabbage, and cauliflower.

Recent studies indicating that AhR activation leads epigenetic regulation of immune response genes open up new avenues to explore the mode of action of dietary AhR ligands.
The Pern-Arnt-Sim (PAS) superfamily of transcription factors is an ancient and highly conserved pathway that regulates communications between the host and the environment and promotes “environmental adaptation”. The PAS superfamily contains proteins that are involved in chemical sensing (AHR), in regulation of circadian rhythm due to light-dark cycles (BMAL1 and BMAL2) and in the detection of variations in oxygen tension or redox potentials (HIF-1α, HIF-2α and HIF-3α).

Naturally Produced Ligands

- Quercetin: (polyphenolic flavonoid)
- Indole 3 Carbazole: (HcL Dependent - cruciferous)
- Benzo(a)pyrene: (polycyclic aromatic hydrocarbons Via cooking)
- Kyneurenine (IDO 2,3 by product of tryptophan)
- 1,4dihydroxy-2-naphthoic acid (Propionibacterium freudenreichii ET-3 isolated from Swiss-type cheese)
Indole-3-carbinol and its condensation derivatives 3,3′-diidolmethane, indolo[3,2-b]carbazole, and 2-(indol-3-ylmethyl)-3,3′-diindolylmethane, are derived from cruciferous vegetables and exhibit strong affinity for the AhR. These or other phytochemicals are very likely to be important in the context of T-cell development, autoimmunity, and inflammation in as much as dietary AhR ligands are critical for the development of gut-associated intraepithelial lymphocytes and maintenance of gut epithelial barrier integrity.
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The Role of the Microbial Metabolites Including Tryptophan Catabolites and Short Chain Fatty Acids in the Pathophysiology of Immune-Inflammatory and Neuroimmune Disease


Effects of microbial metabolites on the production of neuroactive molecules and on the performance of...
By sensing microbial metabolites, the immune system may evaluate microbial activity rather than the mere presence or absence of micro-organisms at a given location. The metabolite profile characteristic of a pathogenic invasion likely differs from the one characteristic of homeostasis, and thus this change in metabolite composition and concentrations may be sensed by the immune system as a deviation from homeostatic set points to initiate an appropriate inflammatory response.
The realization that the immense inter-individual variability in the composition of the microbiota does not allow for the definition of a shared “core” on the taxonomic level (The Human Microbiome Project Consortium 2012). With the study of metabolites as common outputs of bacterial metabolic function, the notion of a functional core can be revisited. It is conceivable that different taxonomic groups of bacteria have evolved to perform similar metabolic functions and are thus classifiable by the type of metabolites that they produce and, consequently, the type of physiologic response that they elicit in the host.

“metabolites are ideally suited as biomarkers for disease development, as their detection is usually affordable and scalable and may precede the onset of clinically manifest disease symptoms.
KEGG pathway map highlighting examples of metabolite modulation of the immune system.

- Taurine
  - NLRP6 inflammasome activation
  - Protection from colitis

- AhR ligands, e.g., indole derivatives
  - Development of ILCs
  - Morphology of intestinal lymphoid follicles
  - Development of IELs

- Polyamines
  - Inhibition of M1 macrophage polarization

- Retinoic acid
  - Alteration of regulatory T cell and T_{h17} differentiation

- Short-chain fatty acids, e.g., acetate, butyrate
  - Induction and maintenance of intestinal regulatory T cells

- Maturation of microglia
- Inhibition of pro-inflammatory gene expression in macrophages

- Epithelial energy source
- Inhibition of autophagy
Certain food additives affect human physiology by altering the gut microbiota. Among the food additives with microbiota-modulating effects are non-caloric artificial sweeteners (NAS), namely, saccharin, sucralose, and aspartame.

Dietary emulsifiers are another group of food additives that affect the intestinal microbiota. Carboxymethyl cellulose (CMC) and polysorbate-80 (P80) are popular dietary emulsifiers that have been approved as GRAS (generally regarded as safe) by the US Food and Drug Administration.
Treatment of SCFA on UC patients has been demonstrated to be effective to ameliorate colitis. SCFA mixture (sodium acetate, sodium propionate and sodium butyrate) enemas, serving as an adjuvant therapy, enhanced the efficacy of classic IBD treatments such as 5-aminosalicylic acid and corticosteroid therapy. **Collectively, SCFA have profound effects on the regulation of gut immunity and the pathogenesis of IBD.**

The discovery that SCFA are the natural ligands for GPR41, GPR43, and GPR109a, which are expressed on a wide range of cell types, has led to re-emerged interest in the role of SCFA in human health, especially in regulation of inflammatory bowel diseases.
Several lines of evidence suggest that brain function and behaviour are influenced by microbial metabolites. Key products of the microbiota are short-chain fatty acids (SCFAs), including butyric acid. Butyrate is a functionally versatile molecule that is produced in the mammalian gut by fermentation of dietary fibre and is enriched in butter and other dairy products.

..we hypothesize that butyrate and other volatile SCFAs produced by microbes may be involved in regulating the impact of the microbiome on behaviour including social communication.
There is ample evidence that production of butyrate by the gut microbiota strongly influences peripheral immune system function, which will in turn shape the brain’s immune milieu.

In addition, butyrate directly affects serotonin and gut hormone release in the enteric nervous system and thereby stimulates the vagus nerve and elicits endocrine signalling, both impacting on brain function.

Alternatively, when artificially administered at high concentrations (>100 mg/kg), butyrate acts as a potent drug with well-established, versatile systemic functions.

It is thus a valuable neuropharmacological agent, most prominently exploited for its HDAC inhibitory potential.
…..diet, microbiota and gut microbial metabolites (particularly SCFAs) can modulate the progression of inflammatory diseases and autoimmunity, and reveal the molecular mechanisms (metabolite-sensing G protein-coupled receptor (GPCRs) and inhibition of histone deacetylases (HDACs)). Therefore, considerable benefit could be achieved simply through the use of diet, probiotics and metabolites for the prevention and treatment of inflammatory diseases and autoimmunity.
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G-protein-coupled receptors (GPCRs) are the largest and most diverse group of membrane receptors in eukaryotes. These cell surface receptors act like an inbox for messages in the form of light energy, peptides, lipids, sugars, and proteins.
G protein-coupled receptors (GPCR) are the largest and most diverse family of transmembrane proteins. They are comprised of 7 transmembrane α-helices, which bind extracellular signals, such as light-sensitive compounds, hormones, growth factors and neurotransmitters, and activate signal transduction pathways inside of the cell, primarily the cAMP and phosphatidylinositol signaling pathways.
...microbe-derived SCFAs and niacin (B3) contribute to the maintenance of gut immune homeostasis by promoting Treg accumulation in the colon through multiple mechanisms.
Butyrate is an attractive therapeutic molecule because of its wide array of biological functions, such as its ability to serve as a histone deacetylase (HDAC) inhibitor, an energy metabolite to produce ATP and a G protein-coupled receptor (GPCR) activator.

Pharmacologically, butyrate has had a profoundly beneficial effect on brain disorders ranging from neurodegenerative diseases to psychological disorders.
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Bacteria as Immune Regulators

Nutrients as Targeted (SNP) Therapy
One explanation for the increased incidence of allergies, asthma, and even some autoimmune diseases has been the hygiene hypothesis. However, recent studies also highlight an important role for diet and bacterial metabolites in controlling various immune pathways, including gut and immune homeostasis, regulatory T cell biology, and inflammation. Dietary-related metabolites engage “metabolite-sensing” G-protein-coupled receptors, such as GPR43, GPR41, GPR109A, GPR120, and GPR35. These receptors are expressed on immune cells and some gut epithelial cells and generally mediate a direct anti-inflammatory effect. Insufficient intake of “healthy foodstuffs” adversely affects the production of bacterial metabolites.
However, relatively little attention has been given to another means of communication between commensal bacteria and the immune system, namely the immunomodulatory effects of microbiota metabolites. These small molecules are intermediates and end products of diet-dependent commensal bacterial metabolism. Many of them serve as functional complementation to the metabolic capacities of the host, providing an example for bona fide mutualistic co-evolution with the mammalian part of the superorganism reducing its genomic capabilities to the extent that can be complemented by the microbiota.

As such, these metabolites integrate the functional states of food intake, microbial ecology and accordingly fine tuning the host response.
In addition to butyrate, de novo Treg-cell generation in the periphery was potentiated by propionate, another SCFA of microbial origin capable of histone deacetylase (HDAC) inhibition, but not acetate, which lacks this HDAC-inhibitory activity. Our results suggest that bacterial metabolites mediate communication between the commensal microbiota and the immune system, affecting the balance between pro- and anti-inflammatory mechanisms.

- Dark leafy greens, vegetables, and grain-like seeds like buckwheat, quinoa, millet, and amaranth.
Apples also help to alter the pathobiont (these are commensals that alter their relationship with the host depending on environmental triggers) mix of bacteria in human guts when consumed regularly; suggesting a role for their use in mild to moderate dysbiosis induced inflammation and loss of tolerance. In a small but clinically interesting study, healthy adults noted an increase in Bifidobacteria species and Lactobacillus numbers also rose, but Clostridium. Perfringens, Pseudomonas and Quantitative PCR was used to measure effects of the polyphenols on the balance between the major groups of intestinal bacteria that are known to influence gut health, i.e., Bifidobacterium spp., Bacteroidetes, and Firmicutes. Fermentation of polyphenols stimulated proliferation of bifidobacteria and decreased the ratio of Firmicutes to Bacteroidetes, relative to controls. …. effect is indirect, i.e., it is mediated by biotransformation products, rather than the original plant compounds.
SCFAs are inhibitors of histone deacetylases (HDACs) and ligands for G protein-coupled receptors (GPCRs), and thereby act as signalling molecules that influence the expansion and function of haematopoietic and non-haematopoietic cell lineages. **SCFA-driven inhibition of HDACs tends to promote a tolerogenic, anti-inflammatory cell phenotype that is crucial for maintaining immune homeostasis, and this activity supports the concept that the microbiota can function as an epigenetic regulator of host physiology.**
The effects of SCFAs are manifold and include **enhanced epithelial barrier function** and **immune tolerance**, which promote gut homeostasis through specific mechanisms: **enhanced production of mucus** by intestinal goblet cells; **inhibition of nuclear factor-κB** (NF-κB); **activation of inflammasomes** and subsequent production of interleukin-18 (IL-18); **increased secretion of secretory IgA** (sIgA) by B cells; **reduced expression of T cell-activating molecules** on antigen-presenting cells, such as dendritic cells (DCs); and **increased number and function of colonic regulatory T (Treg) cells**, including their expression of forkhead box P3 (FOXP3) and their **production of anti-inflammatory cytokines** (transforming growth factor-β (TGFβ) and IL-10).
Butyrate can:
(1) directly affect energy metabolism by acting as a substrate for beta-oxidation;  
(2) can upregulate genes involved in mitochondrial biogenesis (e.g., PGC1α) via its effects as a selective HDAC inhibitor; and  
(3) via its ability to affect the acetylation of a wide number of metabolic proteins.
The proposed mechanisms for the neuroprotective effects of butyrate and the diseases which may benefit from butyrate treatment or a high fibre diet.
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A new example of mitochondria–microbiota functional interaction has been recently published: rats fed with human milk compared with cow’s milk or donkey’s milk display higher energy efficiency associated with change in quality and diversity of their microbiota (Trinchese et al. 2015). These modification of mitochondrial energy metabolism are associated with an increased production of butyrate known to be produced by microbiota and to enter the TCA cycle.

This suggests that diet can modulate mitochondrial function related depending on the quality and diversity of microbiota. All these data suggest that mitochondria play an important role during the interaction of the microbiota with the host cell. Moreover, mitochondrial activity may be an important factor that modulates microbiota diversity and quality, probably due to the role of mitochondria during the inflammatory and immune responses.
…..data suggest that short chain fatty acids not only induce the release of hormones that reduce food intake, but also seem to increase metabolic rate and regulate the immune system and inflammation. This example highlights the mitochondria–microbiota direct interaction for the regulation of energy metabolism.

The perspective will be to modulate the quality and diversity of the microbiota of each person rather than acting on the microbiota metabolites and the microbiota-related factors (NO, H2S, SCFAs). Probiotics, diet or faecal transplantation are new emerging strategies to modulate the quality and diversity of the microbiota.
Reciprocity of Immunity

1. One of the emerging areas of interest is the conflated effect of a dysbiotic gut and locally damaged (dysbiotic) mitochondria, found in epithelial cells, sub mucosal tissues and macrophages.

2. There is a reciprocal activation of local and systemic immune responses – and related Sx.
...people with dysbiosis also develop problems with the membrane integrity of their mitochondria, causing a release of activated enzymes via the triggering of an intracellular environmental switch called the inflammasome. Whilst designed to function as part of our innate defence against microbial infection activated inflammasomes become agent provocateurs and are increasingly linked to persistent inflammation states that lead to a wide range of illnesses.
Often, the same receptors recognize DAMPs and pathogen-associated molecular patterns (PAMPs), which indicates that similarities exist between pathogen-induced responses and non-infectious inflammatory responses.

"STERILE INFLAMMATION"

Review Of Key Actions
Environmental Stressors

- Dysbiosis
- Cytotoxic signals
- Nutrient deficiencies
- Mitochondriopathies
- Cellular aging

Danger Signals

- Mitochondria (Mt)
- MtDNA Ox Cardiolipin release

Inflammasomes (4)

- NOD’s

ROS/RNS

PRR/NOD Activation

- Inflammatory cytokines
  - IL1B & IL-18

Mt Barrier damage

Increased permeabilisation

Mitophagy

Inhibits Mitophagy

CR, LRT, AOX, quercetin, CoQ10 (MitoQ), fibre, apples, probiotics, SB, retinol, vits: D, A, Niacin, exercise, love, cruciferous veg, EFAs, GSH, Curcumin, glutamine, Mtor (resveratrol), Feverfew, meditation………
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Nutrients as Targeted (SNP) Therapy
Maintenance of the epithelial barrier or restoration of a defective barrier is an important component of intestinal homeostasis, and a laudable goal in treating intestinal inflammatory diseases, such as IBD. Yet, despite the fact that the control of paracellular and transcellular permeation is critically energy dependent, the potential to target the epithelial mitochondria to extrinsically regulate gut function has not been addressed.
We suggest that mtROS are an important causative factor in the pathogenesis of IBD. We showed that MitoQ ameliorates acute colonic injury in a mouse model of colitis not only by its antioxidative effects but also by anti-inflammatory effects that suppress the maturation of pro-inflammatory cytokines IL-1 beta and IL-18. Considering the potent protective role of MitoQ in an experimental model of colitis and its proven safety in human clinical trials, MitoQ is a possible therapeutic molecule for the treatment of acute phases of IBD.

NLRP3 Targeting for pathological and functional events related to Dysbiosis
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*The Gastrointestinal Metabolome in Clinical Practice 2016*
Ingested bacteria can temporarily complement resident bacterial communities as part of our transient microbiome. The extent of integration is highly species- and strain-dependent and may vary depending on dietary context and baseline microbiota structure. Ingested bacteria can cause major shifts in the composition of the microbiome of the small intestine, whereas alterations in the colon are mostly of limited extent. Clinical data have provided evidence that ingested bacteria may stimulate production of short-chain fatty acids and inhibit some opportunistic pathogens.

**Fate, activity, and impact of ingested bacteria within the human gut microbiota**

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The human gut contains a highly diverse microbial community that is essentially an open ecosystem, despite being deeply embedded within the human body. Food-associated fermentative bacteria, including probiotics, are major sources of ingested bacteria that may temporarily complement resident bacterial communities as part of our transient microbiome. The extent of integration is highly species- and strain-dependent and may vary depending on dietary context and baseline microbiota structure.

Ingested bacteria can cause major shifts in the composition of the microbiome of the small intestine, whereas alterations in the colon are mostly of limited extent. Clinical data have provided evidence that ingested bacteria may stimulate production of short-chain fatty acids and inhibit some opportunistic pathogens.

Through deeply embedded in, and closely associated with, the human body, the GI tract essentially comprises an open ecosystem. Host genetics plays a role in shaping gut microbiota, but its influence appears to be limited compared to that of environmental factors [7–10], the host's health. Hence, they serve as valuable models for studying the fate of environmental bacteria in the gut microbiome [11]. Here, studies on food-borne pathogens are excluded as these have been reviewed elsewhere and are often associated with pathological host responses that indirectly but strongly impact the gut microbiome and therefore fall outside the scope of this review [12].

**Gut microbiome community structure: core, variable, and transient communities**

Following birth, microbial colonization of the gut involves exogenous bacteria that originate either from the mother's microbiota (mainly from the intestine and vagina) or from other environmental sources. Early colonizers consist mainly of facultative anaerobes that create a favorable niche for more strictly anaerobic bacteria that subsequently dominate the microbiota within a few weeks [16–20]. During the first 3 years of life, radical dietary changes related to weaning, antibiotic use, and modifications in host...
Faecalibacterium prausnitzii is a major commensal bacterium, and its prevalence is often decreased in conditions of intestinal dysbiosis.

Classification of the clone libraries and T-RFLP analysis revealed that Faecalibacterium prausnitzii, reported to be an efficient butyrate producer and a highly metabolically active bacterium in the human intestinal microbiota, was more abundant in the raffinose diet and the chickpea diet compared to the control diet.


The division of bacterial species within the literature into “good” and “bad” organisms is a biologically naive but narratively convenient position that ignores subspecies or strain-specific traits.
Faecalibacterium prausnitzii is the most abundant bacterium in the human intestinal microbiota of healthy adults, representing more than 5% of the total bacterial population. Over the past five years, an increasing number of studies have clearly described the importance of this highly metabolically active commensal bacterium as a component of the healthy human microbiota.
...even though the reported microbial changes in response to interventions are not uniform, the **reduced microbial diversity** in metabolically diseased patients seems to be a fairly recurrent finding. Which bacterial molecules are absent or present in a less diverse microbiota and thereby mediate the development of metabolic diseases is unknown.

**Woting A, Blaut M. The Intestinal Microbiota in Metabolic Disease. Nutrients. 2016 Apr 6;8(4).**
Akkermansia Expansion & Mucin Promoting Foods

- Wheat
- Onions
- Garlic
- Leeks
- Yams
- Chicory root
- Artichoke (the root type, not the spiky globe)
- Agave
- Jicama or Yam
- Bananas
- Dandelion greens
- increased the abundance of *A. muciniphila* by ~100-fold.
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Given that the digestion of foodstuffs primarily occurs in the small intestine, any immune-modulating effects of dietary constituents are likely to show a regionalized effect.

The best examples of such agents are vitamin A (retinol) and ligands of the aryl hydrocarbon receptor (AHR). The only source of vitamin A in mammals is through the diet in the form of plant carotenoids or retinol from animal material, and it is found at higher concentrations in the small intestine.
Although it was originally thought to have a selective role in the induction of tolerance, it is now clear that the effects of retinoic acid on adaptive immunity are context dependent and that it can tune multiple aspects of the immune response.

Thus, this study further demonstrates that provitamin A metabolism is influenced by multiple SNPs and that genetic variability should be taken into account in future recommendations for provitamin A suppletions. (typically retinyl palmitate)

These results illuminate a hitherto unsuspected role of vitamin A in mitochondrial bioenergetics of mammals, acting as a nutritional sensor. As such, retinol is of fundamental importance for energy homeostasis.

The data provide a mechanistic explanation to the nearly 100-yr-old question of why vitamin A deficiency causes so many pathologies that are independent of retinoic acid action.

Compared to a raw carrot meal without avocado, the addition of one avocado (150 g):

- Significantly increased beta-carotene absorption 6.6 times
- More than quadrupled (4.8 times) alpha-carotene absorption
- Significantly increased (12.6 times) the conversion of provitamin A (inactive vitamin form) to vitamin A (active vitamin form)
Reduction in intestinal epithelial VDR levels promotes mucosal inflammation and likely increases the risk of colitis, whereas raising epithelial VDR levels by vitamin D analog therapy or by anti-TNF therapy might have important therapeutic value in the management of IBD. In fact, vitamin D hormone not only can induce VDR expression, but also suppresses TNF-α production. Thus in theory vitamin D therapy can shift the balance to favor inhibition of inflammation and blockade of IEC apoptosis. This could be a mechanism by which vitamin D therapy ameliorates IBD.
vitamin D could influence colonic commensal bacterial profiles through regulation of anti-microbial peptides. Epithelial VDR signaling may also regulate autophagy, another molecular event that has been implicated in IBD. Finally, as a well-known immune regulatory factor, vitamin D-VDR signaling can certainly control mucosal inflammation by regulating the immune system.
....weekly dose of 980 IU/kg bodyweight of vitamin D3 for 4 weeks representing a daily dose of 140 IU/kg bodyweight, but maximal 68,600 IU per week in total. For the remaining 4 weeks, each volunteer received a weekly vitD3 dose of 490 IU/kg bodyweight (daily dose of 70 IU/kg bodyweight), but maximal 34,300 IU per week in total.

140 IU/kg body weight daily (which is close to 11,000 IU for a 180 lb man) for the first 4 weeks and 70IU/kg daily (which is more of a normal dose of 5,500 IU for an 180 lb man) for the last 4 weeks.
Vitamin D3 modulates the gut microbiome of the upper GI tract which might explain its positive influence on gastrointestinal diseases, such as inflammatory bowel disease or bacterial infections. The local effects of vitamin D demonstrate pronounced regional differences in the response of the GI microbiome to external factors, which should be considered in future studies investigating the human microbiome.
The article further proposes that induction of Tregs by probiotics and prebiotics could lead to the development of new therapeutic approach towards curbing the autoimmune response and as an alternative to detrimental immunosuppressive drugs.


“Threshold Therapy”
Key Nutrients Explored

250mg – 1000mg Saccharomyces Boulardii
1 billion – 30 billion Lactobacillus GG, Plantarum, Rhamnosus, Bifido Bifidus
(or other IL10 inducing probiotics)
Iron binding Probiotics
- Propionibacterium freudenreichii/ Escherichia coli Nissle 1917
Diet rich in Ahr ligand foods and SCFA promoting foods
12,000-50,000iu of Vitamin A (Retinol Palmitate)
2,000-50,000iu Vitamin D3
MitoQ 5-10mg daily (or ubiqinol ≥100mg) BID
Membrane Replacement Therapy – 1000mg-1500mg TID
B12 supplementation
Aloe Vera, Oral 50ml, Butyrate
Key Lifestyle Events Explored

Time Restricted Feeding (TRF)
Focused Exercise
Metabolic Integration with Mitochondria
Reduced Exogenous Exposure
Inhibition of Inflammasomes
Microbial Diversification
Metabolite Induction/Modification
xylooligosaccharides (XOS)
Barrier Management
Thank You For Your Attention
The Under-appreciated Role of the Gastrointestinal Metabolome in Innate Detoxification

David Quig, PhD
To be Covered...

• The potentially huge impact of the GI metabolome on innate detoxification processes
• Detoxification of xenobiotic chemical entities
• The influence of the metabolome on inflammation (Nrf2-ARE and NF-κB signaling)
• Potential impact of the metabolome on methionine metabolism
• Urinary 8-OH-dG as a clinically invaluable indicator of *intracellular* oxidative damage
• A two pronged approach to support enhanced glutathione biosynthesis
GI Metabolome- HUGE Player in Detoxification

• The microbiome affects the outcomes of exposures to environmental and food contaminants.

• **Toxicokinetic models** need to be revised to *include* the influence of GI microbial metabolism.

• **Pre-systemic Detoxification** of chemical entities
  
  Conversion a phytoestrogen precursor (hops) to an anti-inflammatory, cardioprotective compound (8-prenylaringenin)\(^1\)

• **Pre-systemic Exacerbation of Toxicants**
  
  *Klebsiella terrigena* converts melamine to cyanuric acid and **exacerbates** melamine-induced nephrotoxicity

  

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GI Metabolome and Detoxification (cont’d)

- **Systemic Biotransformation Processes**
  - inhibition *or* promotion of hepatic cytochrome P450 enzymes (Phase I) - GI epithelium as well
  - inhibition of conjugation reactions (Phase II)

- Toxicants, **stress** and diet can readily **disrupt** the GI microbiome, and thereby affect toxicokinetics and pharmacokinetics.

- **Bottom line:**
  
  The metabolic activity of the GI microbiome rivals, *and* affects that of the liver.


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Detoxification of Chemical Entities

- **Phase I** – “activation”
- **Phase II** – conjugation
- **Phase III** – unidirectional excretion via ATP-dependent efflux pumps
- Optimal detoxification requires *coordinated* regulation of enzymatic activity in *all three phases*

Drug Metab Dispos (2001) 29:779-80
Gram Negative Bacteria-derived Lipopolysaccharide Endotoxins

**Insufficiency dysbiosis**

**Imbalanced Bacteria**

<table>
<thead>
<tr>
<th>Expected/Beneficial flora</th>
<th>Commensal (Imbalanced) flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>2+ Bacteroides fragilis group</td>
<td>2+ Acinetobacter radioresistens*</td>
</tr>
<tr>
<td>1+ Bifidobacterium spp.</td>
<td>2+ Alpha hemolytic strep</td>
</tr>
<tr>
<td>3+ Escherichia coli</td>
<td>1+ Gamma hemolytic strep</td>
</tr>
<tr>
<td>NG Lactobacillus spp.</td>
<td>1+ Pantoea spp</td>
</tr>
<tr>
<td>NG Enterococcus spp.</td>
<td>1+ Staphylococcus aureus</td>
</tr>
<tr>
<td>3+ Clostridium spp.</td>
<td></td>
</tr>
<tr>
<td>NG = No Growth</td>
<td></td>
</tr>
</tbody>
</table>

3+ *Aeromonas caviae*

LPS endotoxin


Trauma Acute Care Surgery(2009)66:1336-42

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LPS Endotoxins Impair Phase I Detoxification

Xenobiotic

Cytochrome P450 isozymes (CYPs)

LPS endotoxin (A. caviae, P. aeruginosa)

Also induces pro-inflammatory cytokine release by GI macrophages, epithelial cells, and systemically.

LPS Activate Inflammatory/Immune Responses

- GI Macrophages, Dendritic and Epithelial Cells
- IBD/gastritis, arthritis, asthma, CVD, obesity, Diabetes type II, neuroinflammation, etc.

Adapter molecules

Transcription factor

activated NF-kB

Microb Ecol Hlth Dis(2012)23 doi 10.3402/mehd.v23i0.19260

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Lactobacilli Activate the Nrf2-ARE Pathway (cytoprotective)

Ubiquitination → Proteasomal degradation

Epithelial NADPH Oxidase 1

Up to 1 million free radicals /sec.

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Nrf2- The Master Antioxidant Regulator

• Nrf2-ARE signaling
  – Promotes epithelial cell proliferation and healing
  – Modifies epithelial NF-κB signaling (pro-inflammatory)

• Activates the “stress proteome”
  Thioredoxin reductase 1, Catalase, NADPH:quinone oxidoreductase-1, GSH peroxidase, Glutamate-cysteine ligase (GCL), GSH reductase, GSH S-transferases (GSTs), UDP-glucuronosyltransferase, metallothioneins, and MRPs

Phase II: Conjugation

• Conjugation of *activated* electrophiles to increase hydrophilicity.

• Some microbial metabolites can impede *sulfonation* and *glucuronidation* reactions.

• *Unconjugated* electrophiles from Phase I can induce *excessive* oxidative stress.

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Phase II: Glutathione Conjugation

Activated Xenobiotic

Pro-oxidative electrophiles, ROS

GSH

Pb, Hg, Cd, As, Co, Cr⁶, Gd

Glutathione S-transferases (GSTs)*

GS-conjugates


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Further Metabolism of GS-conjugates

GS-conjugates

γ-GT (inducible)

γ-GT (inducible)

Cys S-conjugates

N-acetyltransferase

Mercapturic Acids

Phase III

Bile

Intestine

Phase III

Urine

Phase III- Pump it Out

Entails efflux pumps that decrease intracellular levels of Phase II conjugates \textit{(including Hg/As)}

- VERY ENERGY DEMANDING
- MRPs- multidrug resistance proteins
- OATPs-organic anion transport proteins
- Kidney proximal tubule membrane
  Hepatocyte canalicular membrane $\rightarrow$ bile $\rightarrow$ stool

GI Inflammation Impairs Innate Detoxification

• Intestinal and hepatic inflammation down-regulates Phase III efflux pump activity
• Back up of Phases II and I increases oxidative stress
• Clinical Considerations
  Status Beneficial bacteria and SCFA (butyrate- regulator of inflammation)
  Dysbiotic gram negative bacteria (e.g. P. aeruginosa)
  Levels of inflammatory protein biomarkers in stool
  sIgA status (anti-inflammatory effects)
  Vitamin D status (dampens pro-inflammatory cytokine response)
  Serum Zonulin level (influx of proinflammatory macromolecules)

Methionine Metabolism

• Normal methionine (Met) metabolism is essential for: detoxification, neurotransmitter metabolism, immune and mitochondrial functions, and controlling oxidative stress and inflammation.

• Abnormal Met metabolism is associated with-
  – Genetics (SNPs)
  – Epigenetics- nutritional deficiencies and toxicants /endotoxins

• Met is a precursor of S-adenosylmethionine (SAM), cysteine, taurine, glutathione (GSH), and essential sulfate ions


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Methylation
DNA, RNA, protein, neurotransmitters, creatine, phospholipids

Transsulfuration

Transmethylation

Scientific Reports (2015) 5:144-66
Gut-based Impairment of Metabolism

- Dysbiosis-induced Mg wasting/deficiency
  - **MAT requires Mg**
    - **Bacterial dysbiosis** - decarboxylases, aspartate → β-alanine
    - **Yeast overgrowth** - pyrimidine catabolism, uracil → β-alanine
    - **β-alanine** → blocks taurine resorption, and causes taurine and Mg wasting
  - **MAT inactivated** by gut-derived lipopolysaccharides
    - **Oxidative stress** (LPS inhibition of Phases I and III)
    - **LPS-induced TNF-α, IL-6** (via NF-κB signaling)
      - Physiol Rev (2012) 92:1515-42

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The Hydroxyl Radical (\( \cdot \text{OH} \))

- “The most important oxygen free radical causing damage to biomolecules” lipids, proteins and DNA (nuclear and mitochondrial)
  
- Very short T\(^{1/2}\) (10^{-9} seconds)- extremely reactive
  
  - Associated with numerous cancers, CVD, endothelial and mitochondrial dysfunction, inflammatory conditions, and diabetic nephropathy/retinopathy (correlated with HbA1c and BMI)
  
  - Cannot be eliminated by enzymatic reactions or antioxidants

- Intracellular glutathione maintains the redox state of proteins necessary for protection of DNA, and repair and expression of DNA.

24 / 7 / 365 Endonuclease DNA Repair

8-OH-dG levels in 1st AM and 24 hr. urine collections are highly correlated (r=0.93, p<0.01)

DDI unpublished(2012)
Increasing GSH Biosynthesis

- **Rate limiting amino acid** (cysteine)
  - Adequate high BV protein/calories, methionine (folate, B-12, betaine, B vitamins), N-AC, “medicinal” whey protein

- **Induce gene expression of the rate limiting enzyme**
  - γ-glutamylcysteine ligase (GCL)
  - Curcumin (w/piperine), quercetin, oleanolic acid

- Mg, B vitamins

AJCN (2004) 80:1611
Cysteine

\[ \text{Mg, K} \]

\[ \gamma-\text{glutamylcysteine} \]

\[ \text{ATP, glutamine} \]

\[ \text{ADP, Pi} \]

\[ \gamma-\text{glutamylcysteine} \text{ ligase} \]

\[ \text{GSH} \text{ Synthase} \]

\[ \text{Mg, K} \]

\[ \text{GSH} \]

↑ Transcription of GCL
Curcumin
Quercitin
Oleanolic acid
Take Away Points

• **Gut-derived LPS** can activate pro-inflammatory NF-κB that can result in impaired detoxification and excretion of toxicants, increased oxidative stress, and impaired methionine metabolism.

• **Dysbiotic gut-derived β-alanine** can impair retention of taurine and magnesium, and impair optimal methionine metabolism (methylation/transmethylation/transsulfuration).

• Specific commensal bacteria induce Nrf2-ARE signaling that promotes anti-oxidative processes and cytoprotection.

• Urine 8-OH-dG is an excellent indicator of clinically significant *intracellular* oxidative stress... go deep.

• Consider the **two** pronged approach to increase GSH synthesis.
The Impact of the Gut Microbiome on the Brain

8th October 2016
Royal Society of Medicine
Dr. Elisabeth Philipps PhD
Aims of Seminar

• Understand the key bidirectional communication routes between the gut brain axis including:
  
  – Immunological mechanisms
  – Biochemical mechanisms
  – Neuroendocrine mechanisms

• Understand the role of gut microbiota as therapeutically relevant targets in a range of disorders including CFS, MS and CD, as well as more subtle cognitive, psychological and physiological changes
We Are Our Bacteria!

• 26,000 functioning unit in human gene pool compared to 46,000 units in *Oryza sativa* (i.e. rice)!

• “Genome complexity conundrum” speculates that human physiological and behavioural complexity may depend on large number of microbial genes present in the human body

• One hundred trillion bacteria in human adult with 4 million distinct bacterial genes – 95% located in colon

• Most of these genes encode for enzymes and structural proteins that influence mammalian cell function
Gut microbiome can be viewed as “anaerobic bioreactor programmed to synthesise molecules which direct the mammalian immune system, modify mammalian epigenome and regulate host metabolism” (Galland, 2014)
Mechanistic Pathways of the Gut-Brain Axis

Immunological Mechanisms
Through innate and adaptive immune stimulation the gut microbiota can directly modulate brain function including Hypothalamic-Pituitary Adrenal (HPA) axis.
Structural bacterial components and bacterial peptide (e.g. lipopolysaccharides, LPS) translocation:

- Tonically stimulate the innate immune system through pattern recognition receptors (e.g. Toll-Like Receptors) activating inflammasomes and caspase cascade producing pro-inflammatory cytokines (IL-18 and IL-1β) that affect basal GI immune state and microbiota with systemic impact if left unchecked (Guo, 2015)

- NLRP6 inflammasome dysregulation (e.g. from corticotrophin releasing hormone (CRH) mediated stress response) implicated in dysbiosis (and decreased gut epithelial mucin layers), intestinal and neuroinflammation, major depressive disorder and anxiety-like behaviours (Wong, 2016)

- Bacterial peptides also induce intestinal endothelial cells to release neuromodulating molecules acting on primary afferent axons (Dinan, 2015)
**HPA, Inflammation & Gut Microbiota**

- GI bacterial peptide innate immune stimulation alters circulating anti and pro-inflammatory CKs which can directly affect brain function, e.g. IL-1 and IL-6 stimulates hypothalamic release of CRH, a potent peptide regulator of the hypothalamic pituitary adrenal (HPA) axis and stress response

- HPA stress response to elevated pro-inflammatory CKs, salivary and plasma cortisol accompany low mood, increased anxiety, impaired long-term memory, alcohol-cravings and reduced visceral pain sensitivity thresholds

- Low grade LPS-derived systemic inflammation associated with IP, small intestinal bowel overgrowth (SIBO) and low colonisation of GI bacteria with anti-inflammatory effects (Bifidobacterium spp.), as linked to Chronic Fatigue Syndrome (CFS), Restless Leg Syndrome (RLS), fibromyalgia (FM), development of stress-related psychiatric disorders etc.
HPA, Gut Microbiota & Sleep

- Circadian rhythms driven by HPA are also influenced by gut microbiota:
  
  - Sleep studies show gut microbiota stimulation of intestinal macrophages and T-cells to produce CKs (IL-1β/ TNFα) that influence HPA and induce nREM, peaking in the blood at midnight (Cermakian, 2013)
  
  - Natural diurnal cortisol fluctuations induces decline in microbiome-stimulated circulating IL-1β, which may orchestrate normal shift from early nREM sleep to later REM-dominated sleep

- Gut microbes therefore have the potential to shape health and disease including the architecture of sleep and stress reactivity via HPA
Bacterial protein cross reaction with human antigens stimulate dysfunctional adaptive immune system response (i.e. auto-immunity and CNS dysfunction):

- Coeliac disease (CD) alterations in gut microbiome may play a primary role in pathogenesis of GI damage and CNS manifestations, including ataxia, headaches, cognitive dysfunction. Different autoimmune marker detected when gut symptoms are absent (i.e. neurologic CD) – transglutaminase (TG) 6 versus TG2, the target for autoantibodies in commercial tests

- Bifidobacteria levels reduced before CD onset – Bifido (destroyed by anti-biotics) protect human intestinal cells from the toxic effects of gliadin peptides by altering their structure (Laparra, 2010)

- GF diet reduces inflammation but Bifido bacteria do not return to normal levels – B. longum ameliorates animal model of gluten enteropathy (Laparra, 2012)
Biochemical Mechanisms
Intestinal bacteria produce numerous metabolites including D-lactate, ammonia and short chain fatty acids (SCFAs), which can have direct systemic and CNS effects.

Systemic D-Lactate produced by microbial fermentation of carbohydrates (CHOs) – dietary impact on CNS

• Produced in excess in IP, SIBO, abdominal surgery and bacterial translocation

• Increased levels measured in CFS and neurocognitive dysfunction – healing “leaky gut” through diet and supplements reduces levels of systemic D-lactate (Maes, 2007)

• NB. Some Lactobacilli spp. are D-lactate producers! Bifido and FOS favours acetate over lactate as end product of CHO metabolism – understand probiotic supplements!
Ammonia produced from urea by action of intestinal bacterial ureases absorbed into enterohepatic circulation

- Hepatic metabolism maintains systemic ammonia levels – chronic liver damage/ cirrhosis is associated with altered gut microbiome and allows gut-derived ammonia to escape hepatic metabolism increasing blood levels, contributing to hepatic encephalopathy (Quereshi, 2014)

- B. longum and FOS synbiotic mix or mix of non-urease producing bacteria (incl. L. paracasei and L. plantarum) with inulin/pectin reduce serum ammonia and improve cognitive performance in cirrhosis (Zhang, 2013)

- Ammonia has direct neurotoxic insult to blood brain barrier, impairs intracerebral synthesis of 5HT/ DA and produces abnormal NTs like octopamine, affecting subtle intellectual deficits and psychomotor abnormalities
SCFAs (acetate, propionate and butyrate) produced in abundance through bacterial fermentation of indigestible CHO in healthy colon:

- Butyrate supplies 70% colonic epithelial energy requirements – multiple points of action including genes, immunity, inflammation and cell communication

- Propionate has direct anti-inflammatory effects inhibiting NFκβ

- Propionate may improve insulin sensitivity by inactivating peroxisome proliferator-activated receptor gamma

- Increase hippocampal Brain Derived Neurotrophic Factor (BDNF)

- Inhibit histone deacetylation – beneficial effects in cancer, depression, schizophrenia, Parkinson’s Disease

- Activation of CNS G-Protein Coupled Receptors – propionate stimulates GPR41 in human sympathetic ganglia increasing sympathetic outflow and one potential mechanism where fibre can increase basal metabolic rate and control obesity
SCFAs & Autism

• However, some studies show pathological changes in brains exposed to intraventricular propionic acid similar to abnormalities in children and adults with autism presenting with neuroinflammation, depleted glutathione and increased markers of oxidative stress (butyrate similar effects but milder) (Macfabe, 2013)

• Elevated SCFA and propionate in faecal samples from autistic children (Wang, 2010 & 2012) – autism is associated with early weaning from breast milk to infant formula; compared to breast milk, infant formula increases faecal concentrations of propionate and butyrate (Macia, 2006)

• SCFA-induced neurotoxicity may partly explain sensitivity of autistic children to dietary CHO with increased fermenting gut bacteria populations (e.g. Clostridial spp.)

• Majority of children with positive biopsies also had reactivity to Sutturella wadsworthensis - GI pathogen often mistaken for Campylobacter jejuni (Wang, 2013)

• Brain abnormalities may even occur in utero (immune activation of pregnant mice can create behavioural changes similar to ASD in offspring reduced by Bacteroides fragilis administration) suggesting importance of maternal gestational microbiome, newborn care and parental education (Muelle, 2013)
Neuroendocrine Mechanisms
Gut microbiota can directly stimulate of ENS & CNS afferent neurons, as well as produce and respond to hormones and neurotransmitters.
Gut microbiota and their secretions directly influence neuronal activation in the ENS, regulating gut motility and sensory afferent signalling to the brain via vagal nerve.

- Vagal-independent neuronal activation may also occur as CNS levels of BDNF can be altered in mice by manipulation of the gut microbiome without vagal involvement (Bercik, 2011).
Gut microbiota can synthesise and respond to hormones and neurotransmitters (NTs)

- Lactobacilli spp. produce ACh, GABA; Bifido produce GABA; Escherichia produce NA, 5HT and DA; Strep and enterococcus produce 5HT; Bacillus produce NA and DA – see Antony’s NeuroBiogenic Amines presentation (www.nleducation.co.uk)

- Studies using germ-free mice, antibiotics and probiotics, infections and fecal transplantation all show differing levels of NT plasma metabolites (including DA and 5HT) depending on gut microbiota composition

- Accumulating evidence for key functions of microbial genes in neuronal function for the developing and mature brain (Dinan, 2015)

- Gut bacteria also respond to host NTs impacting their growth and virulence – E. coli growth increases through NA exposure so stress can increase infection, independent of the effect of stress on host immunity (Freestone, 2008)
Microbiota, Mood & Memory

• L. casei improved mood within 3 weeks for elderly men and women (Benton, 2007)

• Ingestion of strain specific B. breve in mice improved cognitive processes such as memory and learning (Wall, 2012) – can we translate this to Alzheimer's Disease where elderly are seen with lowest diversity in gut microbiota? (Claesson, 2011)

• Interrelated probiotic mechanisms include (Dinan, 2015):
  – Decreased IP with reduced LPS absorption and pro-inflammatory CK production
  – Down regulation of HPA axis responding to stressors
  – Direct effects on neurotransmission
Gut Microbiota & Tryptophan

- Gut microbiota influences tryptophan (TRY) metabolism (essential AA), ENS/CNS serotonin (5HT) levels and serotonergic system, which regulates gut motility and mood – dysregulated in many digestive and brain disorders (O’Mahony, 2015)

- Dietary metabolite TRY acts as transcription factor agonist at aryl hydrocarbon receptors (AhR) on astrocytes to limit CNS inflammation

- Manipulate healthy and functional microbiota capable of manufacturing metabolites for strategic clinical route? e.g. MS patients with lower TRY-derived AhR serum ligands (Reber, 2016)
**Tryptophan Metabolite Neuroendocrine Pathways**

- Inflammatory mediators (e.g. cortisol, bacterial LPS, CKs) stimulate GI and hepatic TRY metabolism to kynurenine lowering systemic levels of 5HT (Dantzer, 2008)

- Kynurenic acid acts as NMDA receptor antagonist associated with anti-excitatory activity but cognitive degeneration, as seen in schizophrenia and cognitive degeneration at elevated levels (Javitt, 2014)

- Quinolinic acid downstream product of kynurenine is NMDAR agonist with potent neurotoxic effects and also known to alter the integrity and cohesion of the BBB (Guillemin, 2012)
Understanding the direct mechanisms involved in gut-brain axis pathways helps to formulate a therapeutic approach.
Summary

• Understanding the interrelated mechanisms of gut-brain axis function and dysfunction via immune system, biochemical and neuroendocrine effects allows for manipulation of gut microbiome using diet and clinically relevant GI nutritional supplements.

• Gut microbes exist in a series of interconnected and highly structured living communities - administering a probiotic or supporting gut microbiome does more than just introduce new bacterial species.

• Gut microbial composition varies among individuals dependent on age, genetic background, physiological state, microbial interactions, environmental factors, diet.

• Over to Antony to place the mechanistic knowledge into clinically relevant framework!


• Williams BL, Hornig M, Buie T, et al.: Impaired carbohydrate digestion and transport and mucosal dysbiosis in the intestines of children with autism


Gastrointestinal Metabolome in Clinical Practice:
Clinical Application, Case Histories & likely mechanisms

8th October 2016 Royal Society of Medicine
Antony Haynes BA(Hons), Dip ION
Registered Nutritional Therapist
Case Reports: Using Fxmed Principles & Practice.

1 - Andrea
• 38 year old lady: 8 years of extreme fatigue, brain fog, complete loss of libido, & digestive symptoms - resolve within a week with targeted Nutritional Therapy (NT). Using Fxmed principles and practice.

2 - Fiona
• 17 year old young woman: acute & stabbing gut pain, fatigue, disrupted sleep - all resolve with targeted NT.

3 - Sarah
• 57 year old woman suffering from declining memory & altered cognitive function, insomnia, aching joints & menopausal symptoms that all resolve with targeted NT.

4 - Tom
• 7 year with ADHD whose motor skills, spatial development, concentration & energy all improve with targeted NT.
Clinical Applications – Case Reports

• Case Report title
• Case History Summary & Health Goals
• Nutritional Therapy Intervention
• Client outcome
• Summary Explanation of Likely Mechanisms
Case One

Miss A.P. – “Andrea”

38 year F whose 8 years of extreme fatigue, brain fog, loss of libido, & digestive symptoms resolve within a week with targeted NT.
Case History Summary – Health Goals

1. To have a healthy functioning gut
2. To be free of bloating & gas
3. To have normal blood sugar balance
4. To be free of exhaustion & have great energy & vitality
5. To get my sex drive back
6. To be free of seasonal episodes
Mind Maps Colour Code

- **Bold Black** = current relevant information
- Black (not bold) = current health information
- Blue = possible relevant diagnoses or conditions
- **Orange** = food recommendations, influence of food
- **Green** = supplement intervention
Andrea (38)

- Hard work
- Bike Accident
- Head Injury 2007
- Burn Out?
- ‘PTSD’
- Poor Mood
- Aches & Pains
- ? CFS / FM ?
- Unrefreshing Sleep
- Extreme Fatigue
- Inflammation
- Brain Fog
- ? S.A.D. ?
- No Libido
- Uncontrollable Food Binges
- Wind Bloating Pain Constipation
- Food Poisoning in Bali 2015
- Adrenals
- Grain Free Diet
- Oregano Extract
- Free Form Aminos
- Sufficient Protein
- Thicker Stronger Hair
- Sugar
- Banaba Leaf Extract
- Bloating
- Pain
- Constipation
- Inflammation
- Free Aminos
- No Sugar
- Thicker Hair
Nutritional Therapy Intervention - Andrea

**Dietary Changes**

- Protein with each meal
- Relax before eating & chew food well
- Cooked veg (not raw)
- No grains but alternatives provided

**Supplemented Nutrients**

- Emulsified, sustained release oregano extract
- Free form amino acids
- Trace lithium
- Banaba leaf extract providing Corosolic acid (supports cellular glucose disposal)
Client Outcome - Andrea

• “All gut issues disappeared ‘literally overnight’, all energy and blood sugar issues, brain fog, slight negativity also vanished. For the first time in 8 years I feel a desire to partake in life”.

• My hair is thicker.
• My bowels are better.
• Inertia & brain fog has disappeared.
• Sex drive improved.
• Aches & pains vanished.
Summary Explanation of Likely Mechanisms

Andrea

• Immunological changes due to oregano extract inhibiting a range of microbes, improving balance of microbiota & reducing inflammation.
• Subsequent reduction in inflammation led to improved bowel activity.
• Improved enteric/central nervous system function due to trace lithium, probably improving parasympathetic dominance, may have contributed to improved bowels.
• With reduced inflammation in the gut, due to reduced immune activity, the enteric nervous system messaging to the CNS directly and via neurotransmitter receptor function improved, and no longer interrupted or interfered with. As a result, inertia was overcome!
• Hair is thicker due to protein & amino acid availability, & suppressive effect of stress on anabolic processes being corrected, maybe, and possibly suppression of thyroid hormones was alleviated.
• Sex drive improved due to energy improvement which is likely due to improved blood glucose balance and sufficient protein, & reduction of inflammation.
• Aches & pains vanished due to reduced inflammation due to change in microbiota.
Case Two

Miss F.M. – “Fiona”

17 year old F, with stabbing gut pains, fatigue, disrupted sleep all resolve with targeted NT.
Case History Summary – Health Goals

1. To be free of stabbing gut pains.
2. To sleep through the night.
3. To feel good, to be in good mood.
4. To have good energy all day, every day.
5. To be free of burping.
Mind Maps Colour Code

- **Bold Black** = current relevant information
- Black (not bold) = current health information
- Blue = possible relevant diagnoses or conditions
- Orange = food recommendations, influence of food
- Green = supplement intervention
Fiona (17)

- Menstruation
- PMT
- H. Pylori ?
- SIBO ?
- History of Antibiotics Aged 9
- Pale Complexion
- Poor mood: flat
- Fatigue
- Stabbing Gut Pains
- Pain Killers
- Period Pains
- Inflammation
- “IBS”
- Bloating Burping Constipation Diarrhoea
- Antidepressants
- Fatigue
- Resilience Supplement
- Active B Vits
- Post Elimination Diet Crash
- Diverse Diet
- Hot Water Bottle
- Dairy Products
- Wheat
- Fructose Intolerance
- Wheat
- Diverse Diet
- Hot Water Bottle
Nutritional Therapy Intervention - Fiona

Dietary Changes

• Avoidance of wheat, dairy, fructose & sugar.
• Increased water intake.
• Healthy alternatives recommended, reconstruction of diet.

Supplemented Nutrients

• Perilla extract Benegut® with modified citrus pectin and traditional botanicals, including bromelain, papaya, cayenne, ginger, fennel seed, oregano, and chlorophyll.
• Ling fish extract as adaptogen
• Low dose, active B vit formula
• then Evening Primrose Oil
Client Outcome - Fiona

- Brief euphoria, then crash (flu-like symptoms & headache)
- Then gradual improvement in energy
- Stabbing pains subside
- Gut symptoms start to resolve
- Outbreak of spots
- Worse menstrual pains
- Better sleep, more refreshed a.m.
Client Outcome ii - Fiona

- Improvements in energy
- Much less menstrual pain
- Over time, free from all GI symptoms
Summary Explanation of Likely Mechanisms - Fiona

• Brief euphoria, then crash is a pattern that can occur when avoiding wheat or gluten or a food to which an immunological response was occurring. Immediate reduction in immune & inflammatory responses followed by an elimination process of antigen-antibody complexes.

• Energy improves as a result of reducing burden of inflammatory complexes & reduced stress hormones & improved blood glucose balance.

• Stabbing pains subside due to omission of foods which previously triggered immune mediated inflammatory response within the gut.

• Gut symptoms lessen due to reduced immune mediated inflammatory reactions, & improved ENS neurotransmitter production and receptor function in the gut.

• Outbreak of spots likely due to a detoxification process within the body of historical antigen-antibody complexes which increases some systemic cytokine levels. Not sure if androgens are involved.

• Intensified menstrual pains possibly due to a detoxification process within the body of historical antigen-antibody complexes which increases some systemic cytokine levels.

• Better sleep, more refreshed a.m. due to reduced CNS irritation by ENS, reduced immunological activity due to food eliminations, improved blood glucose balance, reduced stress hormones & improved neurotransmitter levels and functions.
Case Three

Mrs S.N. – “Sarah”

57 year old F, suffering from a declining memory & altered cognitive function, insomnia, aching joints & menopausal symptoms that all resolve with targeted NT.
Case History Summary – Health Goals

1. To improve my memory
2. To be free of brain fog & regain my mental clarity
3. To be free of pain
4. To feel stronger and younger
5. To sleep well through the night
6. To be free of menopausal symptoms
Mind Maps Colour Code

- **Bold Black** = current relevant information
- Black (not bold) = current health information
- Blue = possible relevant diagnoses or conditions
- **Orange** = food recommendations, influence of food
- **Green** = supplement intervention
Sarah (57)

- Rapid Worsening Memory & Cognitive Function
- Age of 57
- Inflammaton
- Protein?
- Very Busy Life
- Interrupted Sleep
- Adrenals
- Post Menopausal Symptoms
- Peeing in the night

- Past Riding Injuries
- Physical Strength
- Liver
- Increasingly Achy Joints

- Collagen Powder
- LDL Cholesterol
- Vit D & A & K2 & Tocotrienols
- Lactobacillus Rhamnosus

- Food Reactivity
- Gluten
- Vit B1, B5, methyl donors & CoQ10

- Herbal extracts incl cone hops extract

- Hotel Reaktivy
- Glue
- LDL Cholesterol
- Vit D & A & K2 & Tocotrienols
- Lactobacillus Rhamnosus
Nutritional Therapy Intervention - Sarah

**Dietary Changes**
- Gluten Free Diet
- Grain Free Diet

**Supplemented Nutrients**
- Lactobacillus rhamnosus GG
- Collagen powder (hydrolyzed)
- Mixed Tocotrienols
- Vit D with A, E, & K2
- B1 & B5 with methyl donors & CoQ10
- Herbal extract formula for female hormone balance
Client Outcome - Sarah

• Rapid improvement in brain function
• Gradual reduction in aches & pains
• Gradual improvement in energy
• Less disrupted sleep
• Improved vaginal dryness
• Absence of hot flushes
Immunological Mechanisms

Biochemical Mechanisms

NeuroEndocrine Mechanisms
Summary Explanation of Likely Mechanisms Sarah

- Rapid improvement in brain function likely due to gluten-induced brain hypofusion correction, combined with likely reduced GI immune mediated inflammatory activity.
- Gradual reduction in aches & pains due to reduced immune activity and subsequent inflammation from the gut decreasing systemic cytokine burden.
- Gradual improvement in energy due to reduced GI immune mediated inflammation combined with improving hormonal efficacy.
- Less disrupted sleep due to reduced GI immune mediated inflammation, with consequent improved neurotransmitter function & reduced stress hormone response to the inflammation, and more balanced energy hormones (adrenals and thyroid) & improved HPA due to reduced immunological interactions.
- Improved vaginal dryness due to the herbal extracts, possibly aided by reduced overall inflammatory burden.
- Absence of hot flushes due to the herbal extracts, possibly aided by reduced overall inflammatory burden.
Case Four

Master T.H. – “Tom”

7 year M, with ADHD whose motor skills, spatial development, concentration & energy all improve with targeted NT.
Case History Summary – Health Goals

1. To improve concentration
2. To improve energy
3. To improve motor skills
4. To fall asleep fast & sleep well through the night
5. To be free of blocked sinuses & chesty coughs
6. To gain healthy weight
7. To identify an optimal diet for Tom
8. To identify what supplements are needed
9. To avoid the need for Ritalin
Mind Maps Colour Code

- **Bold Black = current relevant information**
- **Black (not bold) = current health information**
- **Blue = possible relevant diagnoses or conditions**
- **Orange = food recommendations, influence of food**
- **Green = supplement intervention**
Tom (7)

ADHD
Motor Skills
Spatial Awareness
Concentration / Focus

Delayed Development

Small for his age

Agitated at Bedtime

Disrupted Sleep

Energy

MRT
Phospholipids

? Inflammation ?

? Inflammation ?

? Inflammation ?

Active B Vits

Adrenals ?

Cranial Osteopathy

Neurotransmitter Balance

Occupational Therapy

Digestive Enzymes

Food Reactivity

Wheat
Cow’s Products
Orange Juice

Natural Mucolytic

Blocked Sinuses
Chesty Coughs

Energy

Wheat
Cow’s Products
Orange Juice

Blocked Sinuses
Chesty Coughs
Nutritional Therapy Intervention - Tom

**Dietary Changes**
- Elimination diet: no wheat, no cow’s products and no orange juice (& oranges).
- Replacements & alternatives found, resulting in more diverse diet.

**Supplemented Nutrients**
- Phospholipids for Membrane Replacement Therapy & reduction of fatigue
- Active, low dose B vits
- Digestive enzyme incl glutenase
- Lactobacillus rhamnosus GG
- Natural mucolytic agent
Client Outcome - Tom

Within 5 weeks
Energy & concentration improved
Minor weight gain
Spatial & physical improvements

Later
Mucus improved
Free from infections
Improved sleep
Immunological Mechanisms

Biochemical Mechanisms

NeuroEndocrine Mechanisms
Summary Explanation of Likely Mechanisms

Tom

- Energy & concentration improved primarily due to omission of foods that elicited an immune mediated inflammatory response, and possibly by key nutrients (MRT & active B vits) & possibly by improved digestion (enzymes).
- Minor weight gain due to reduced immune mediated inflammation which has a catabolic effect, as well as subsequent improved digestion & absorption from increased diversity of diet & enzymes & probiotics.
- Spatial & physical improvements due to reduced immune mediated inflammation and subsequent improved neural messaging along with provision of key nutrients for the nervous system (MRT & active B vits).
- Mucus improved due to natural mucolytic agent.
- Free from infections due to less mucus, reduction in immune challenge in gut, and improved immune tolerance supported mostly by food elimination & less so by provision of key energy nutrients.
- Improved sleep due to reduced overall body burden of inflammatory cytokines (immune reactivity to foods), improved nutrient level, more optimal level of key energy enzymes due to active B vits & mitochondrial ATP levels due to MRT.
Many Thanks!

For your kind attention