OVERVIEW OF THE DIGESTIVE, SENSING, BARRIER, AND IMMUNE FUNCTIONS OF THE GUT

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DISCLOSURE OF FINANCIAL RELATIONSHIPS

• Ortho Molecular Products
  • Consulting Fees
• Genova
  • Speaker Fees
• Off-Label Usage
  • None
LEARNING OBJECTIVES

• Understand the nomenclature and framework of GI functions as a foundation to an integrative approach to successful therapies.

• Appreciate the inter-relationship between the various GI functions and how those functions support (or create vulnerabilities) for one another.

• Review simple methods of evaluating basic digestive, barrier and microbial environmental function of the GI tract

• Understand the importance of the GI immune system and the necessary education (tolerance) it provided for the whole immune system
ANOTHER WAY TO DESCRIBE THE CORE FUNCTIONS OF GI

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THE CHALLENGE OF DIGESTION & ABSORPTION

- Breakdown complex foods into basic constituents
- Divide Macronutrients into basic units
- Release Micronutrients from food matrix
- Selectively absorb nutrients
- Transform and/or activate nutrients and phytonutrients
- While maintaining a barrier against entry of unwanted particles
Nutrients are not absorbed uniformly, there are specific locations where the available transporters or necessary processes are found.

Appropriate timing and sequence is also important- bowel transit time can adversely affect this.

Transporters and enzyme capacity can be overwhelmed/saturated, reducing the effective benefit of dietary nutrients.

Nutrients produced by colonic bacteria may have limited human bioavailability, though may benefit colonocytes and microbes.
DIGESTION AND ABSORPTION
WHAT CAN GO WRONG?

Poor Digestion & Absorption

- Poor Dietary Habits:
  - Food selection
  - Food preparation
  - Meal timing
  - Poor chewing

- Altered Bowel Transit Time

- Villous Atrophy
  - Brush-border enzymes and transporters

- Dysbiosis

- Altered Gut/Neuroendocrine Signaling

- Low Endogenous Levels of:
  - Stomach acid
  - Saliva
  - Pancreatic Enzymes
  - Bile

- Pharmaceutical Agents
  - Acid-blocking
  - Nutrient inhibition

- Reduced Bioavailability of Nutrients and Bio-active Ingredients to Tissues (GI and Systemic Symptoms)
  - Potential deficiency-related outcomes
  - Reduced metabolic efficiency
  - Altered genomic activation
  - Altered epigenetic signaling

- Increased Availability of Undigested and/or Un-neutralized Food Particles ( Mostly GI Symptoms)
  - ↑ Allergenicity/immunogenicity
  - ↑ Inflammatory triggers
  - ↑ Burden for detoxification
  - ↑ Fermentation and putrefaction via gut microbiota

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DIGESTION AND ABSORPTION
THE CEPHALIC PHASE

Stimuli
- Conditioned reflexes
- Sensory input (e.g., sight, smell, auxiliary taste)
- Thought of food
- Chewing
- Swallowing
- Hypoglycemia

Stomach
- Stimulation of gastric secretions. See Figure X for detailed explanation of gastric secretion regulation.

Mouth
- Stimulation of salivation

Gallbladder
- Stimulation of mild gallbladder contraction, sphincter of Oddi remains closed.

Pancreas
- Stimulation of enzyme and bicarbonate secretion

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<table>
<thead>
<tr>
<th>Lumen of stomach</th>
<th><strong>Cell Types</strong></th>
<th><strong>Substance Secreted</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mucous neck cell</td>
<td>Mucus (protects lining)</td>
</tr>
<tr>
<td></td>
<td>Parietal cells</td>
<td>Bicarbonate</td>
</tr>
<tr>
<td></td>
<td>Enterochromaffin-like cell</td>
<td>Gastric acid (HCl)</td>
</tr>
<tr>
<td></td>
<td>Chief cells</td>
<td>Intrinsic factor (Ca++ absorption)</td>
</tr>
<tr>
<td></td>
<td>D cells</td>
<td>Histamine (stimulates acid)</td>
</tr>
<tr>
<td></td>
<td>G cells</td>
<td>Pepsin(ogen)</td>
</tr>
</tbody>
</table>

|                 | Gastric lipase |
|                 | Somatostatin (inhibits acid) |
|                 | Gastrin (stimulates acid) |

**PYLORIC GLAND**

- **GASTRIC PIT** (parietal zone)
- **ISTHMUS** (intermediate zone)
- **NECK**
- **BASE**

**OXYNTIC GLAND**

- **GASTRIC PIT** (parietal zone)
- **ISTHMUS** (intermediate zone)
- **NECK**
- **BASE**
Gut epithelium showing different representative enteroendocrine cell (EEC) types. (a) Gastric somatostatin-producing D-cell with basolateral process that communicates with (b) a neighboring gastrin-producing G-cell. (c) Closed-type EEC and (d) small intestinal/colonic-type open EEC with neuropod basolateral extension.
Table 1  Key hormones, possible secretory stimuli, and physiological processes occurring along the gut axis

<table>
<thead>
<tr>
<th>Gut region</th>
<th>Intestinal processes</th>
<th>Luminal stimuli of EECs</th>
<th>Principal gut hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>Acid secretion</td>
<td>Acid</td>
<td>SST, histamine, 5-HT, ghrelin, gastrin</td>
</tr>
<tr>
<td></td>
<td>Mechanical disruption</td>
<td>Digested protein</td>
<td></td>
</tr>
<tr>
<td>Duodenum</td>
<td>Release of bile acids, pancreatic and intestinal enzymes, bicarbonate Digestion Absorption</td>
<td>Monosaccharides Free fatty acids Monoacylglycerols Amino acids Di/tripeptides Bile acids</td>
<td>Duodenum: GIP, ghrelin, CCK, 5-HT, SST</td>
</tr>
<tr>
<td>Jejunum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proximal ileum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Terminal ileum</td>
<td>Bile acid reabsorption</td>
<td>Bile acids Unabsorbed nutrients</td>
<td>GLP-1, GLP-2, PYY, Nts, 5-HT</td>
</tr>
<tr>
<td>Colon</td>
<td>Bacterial metabolism</td>
<td>Short-chain fatty acids Indole Secondary bile acids</td>
<td>GLP-1, GLP-2, PYY, Nts, Ins15, 5-HT</td>
</tr>
<tr>
<td>Rectum</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The details are based on mouse data and are discussed in the text. Abbreviations: 5-HT, 5-hydroxy-tryptamine (serotonin); CCK, cholecystokinin; EECs, enteroendocrine cells; GIP, glucose-dependent insulinotropic polypeptide; GLP-1 and GLP-2, glucagon-like peptides 1 and 2; Ins15, insulin-like peptide 5; Nts, neurotensin; PYY, peptide YY; SST, somatostatin.
CONTROLLING ACID PRODUCTION

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HOW MUCH ACID DO WE NEED?

• Low Stomach acid contributes to:
  • Reduced protein digestion (denaturing)
  • Increases protein allergenicity
  • Reduced solubility/absorption of key nutrients like calcium, iron, folic acid, vitamins B6 and B12
  • Increases SIBO, C. diff and related harmful bacteria

• But how low is too low?
  • This is a very debatable question and one that is not well studied…..
MEASURING STOMACH ACID:

- Heidelberg radio-telemetric capsule
  - Best used for alkali challenge/reacidification

Some research also uses pH sensitive tablets and follow collection of metabolites in urine (i.e. riboflavin).
HYPOCHLORHYDRIA AND ACHLORHYDRIA

• It is generally assumed that **fasting pH** below 3.0 is considered “normal”
• Achlorhydria (no stomach acid) results in fasting pH of about 7 (neutral) or above.
• This is common in subjects with atrophic gastritis.
• Fasting Hypochlorhydria is considered to be present in about 10% of the aging American population, though upwards of 60% in older Japanese adults.
• Low stomach acid is not uncommon in patients with GERD-like symptoms
GERD IS NOT ASSOCIATED WITH EXCESSIVE ACID PRODUCTION!

Control Subjects (N=54)

Subjects with GERD (N=1,582)

FUNCTIONAL HYPOCHLORHYDRIA

- Our body secretes acid primarily to digest food, therefore, fasting levels are not nearly as important as prandial levels (when you eat) - measured by re-acidification after eating food.

- If fasting gastric acid production goes down with aging, what happens to gastric pH during a meal?

DOES THIS REPRESENT A FUNCTIONAL DIFFERENCE?
OLDER SUBJECT TAKE LONGER TO ACIDIFY STOMACH AFTER A MEAL!

Table I. Comparison of Gastric pH Between Young and Elderly Subjects

<table>
<thead>
<tr>
<th>Treatment phase</th>
<th>Young ( (N = 24)^a )</th>
<th>Elderly ( (N = 79)^b )</th>
<th>( P ) value(^c )</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fasted</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median pH(^d)</td>
<td>1.7 (1.4–2.0)</td>
<td>1.3 (1.1–1.6)</td>
<td>0.014</td>
</tr>
<tr>
<td>AUC (pH + hr)</td>
<td>2.0 (1.6–2.4)</td>
<td>1.4 (1.2–1.9)</td>
<td>0.006</td>
</tr>
<tr>
<td>( N = 24 )</td>
<td>( N = 75 )</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>During the meal</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median pH</td>
<td>5.0 (4.4–5.6)</td>
<td>4.9 (3.9–5.5)</td>
<td>0.74</td>
</tr>
<tr>
<td>Peak pH</td>
<td>6.6 (6.3–7.0)</td>
<td>6.2 (5.8–6.7)</td>
<td>0.02</td>
</tr>
<tr>
<td><strong>Postprandial</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Time to return to pH 5 (min)</td>
<td>8 (2–17)</td>
<td>23 (6–46)</td>
<td>0.015</td>
</tr>
<tr>
<td>Time to return to pH 4 (min)</td>
<td>14 (8–46)</td>
<td>52 (27–115)</td>
<td>0.0002</td>
</tr>
<tr>
<td>Time to return to pH 3 (min)</td>
<td>42 (26–83)</td>
<td>89 (44–167)</td>
<td>0.0026</td>
</tr>
<tr>
<td>Time to return to pH 2 (min)</td>
<td>100 (44–143)</td>
<td>154 (82–210)</td>
<td>0.026</td>
</tr>
<tr>
<td>AUC (pH + 4 hr)</td>
<td>10.8 (8.1–12.2)</td>
<td>12.3 (8.6–15.3)</td>
<td>0.0001</td>
</tr>
<tr>
<td>( N = 24 )</td>
<td>( N = 78 )</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
THE PANCREAS: MULTIPURPOSE GLAND

trypsin, chymotrypsin, elastase, pancreatic lipase, pancreatic amylase

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### Enzymes of the Human Exocrine Pancreas

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Proenzyme</th>
<th>Activator</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trypsin</td>
<td>Trypsinogen</td>
<td>Enteropeptidase</td>
<td>Cleaves internal peptide bonds</td>
</tr>
<tr>
<td>Chymotrypsin</td>
<td>Chymotrypsinogen</td>
<td>Trypsin</td>
<td>Cleaves internal peptide bonds</td>
</tr>
<tr>
<td>Elastase</td>
<td>Proelastase</td>
<td>Trypsin</td>
<td>Cleaves internal peptide bonds</td>
</tr>
<tr>
<td>Carboxypeptidase</td>
<td>Procarboxypeptidase</td>
<td>Trypsin</td>
<td>Cleaves last amino acid from carboxyl-terminal end of polypeptide</td>
</tr>
<tr>
<td>Phospholipase</td>
<td>Prophospholipase</td>
<td>Trypsin</td>
<td>Cleaves fatty acids from phospholipids such as lecithin</td>
</tr>
<tr>
<td>Lipase</td>
<td>None</td>
<td>None</td>
<td>Cleaves fatty acids from glycerol</td>
</tr>
<tr>
<td>Amylase</td>
<td>None</td>
<td>None</td>
<td>Digests starch to maltose and short chains of glucose molecules</td>
</tr>
<tr>
<td>Cholesterolesterase</td>
<td>None</td>
<td>None</td>
<td>Releases cholesterol from its bonds with other molecules</td>
</tr>
<tr>
<td>Ribonuclease</td>
<td>None</td>
<td>None</td>
<td>Cleaves RNA to form short chains</td>
</tr>
<tr>
<td>Deoxyribonuclease</td>
<td>None</td>
<td>None</td>
<td>Cleaves DNA to form short chains</td>
</tr>
</tbody>
</table>

- Note the importance of trypsin in cleaving several precursor enzymes (zymogens) into their final active form.
- Note also that trypsinogen is first cleaved by enteropeptidase (enterokinase)- an enzyme located on the surface of duodenal cells- requiring an intact brush border system.
IMPORTANCE OF BRUSH BORDER ENZYMES
LIPID TRANSPORT INTO CIRCULATION

- Fat globules (lipids) + Emulsion droplets
  - Digestion by lipases → Free fatty acids (monoglycerides) + Micelles
  - Bile salts
  - Bile salts

- Chylomicrons
  - Triglyceride
  - Secretory vesicle
  - Protein

Copyright © 2001 Benjamin Cummings, an imprint of Addison Wesley Longman, Inc.
LOW PANCREATIC ENZYME OUTPUT

- Pancreatic Exocrine Insufficiency: defined as output below 10% of normal (i.e. 90% reduction).
  - Common in chronic pancreatitis, cystic fibrosis, pancreatic cancer
  - Also caused by: gastrectomy, gastric bypass or GI tract disorders like celiac disease…and aging!
- Measured by:
  - Fecal Fat Analysis
  - Radiolabeled TG test
  - Or Pancreatic Elastase 1 (a.k.a. fecal elastase)
<table>
<thead>
<tr>
<th>Analyte, Related Profiles</th>
<th>Result</th>
<th>Suspect</th>
<th>Consider</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chymotrypsin</td>
<td>Low &lt; 0.9 mcg/g</td>
<td>Pancreatic insufficiency or hypochlorhydria</td>
<td>Assess proteotoxic SCAs &lt;br&gt; Therapeutic interventions &lt;br&gt; Pancreatic enzyme supplementation and/or betaine HCL &lt;br&gt; Dietary fiber (insoluble) to improve transit time</td>
</tr>
<tr>
<td></td>
<td>Normal 0.9-26.8 mcg/g</td>
<td>Adequate exocrine pancreatic function</td>
<td>1-2 SD = Results from 1-2 SD (yellow range) warrant clinical correlation even though within the “normal” reference range.</td>
</tr>
<tr>
<td></td>
<td>Elevated &gt; 26.8 mcg/g</td>
<td>Rule out false elevations from diarrhea (assess pancreatic elastase 1 levels) &lt;br&gt; Further Testing: &lt;br&gt; Comprehensive Parasitology Profile &lt;br&gt; Bacterial Overgrowth of the Small Intestine &lt;br&gt; Lactose Intolerance &lt;br&gt; Food Antibody Assessment &lt;br&gt; Celiac Testing</td>
<td></td>
</tr>
<tr>
<td>Pancreatic Elastase 1 (PE1)</td>
<td>Low 100-200 mcg/g</td>
<td>Mild to moderate pancreatic insufficiency</td>
<td>Further Testing: &lt;br&gt; Intestinal Permeability Assessment &lt;br&gt; Comprehensive Parasitology Profile &lt;br&gt; Celiac Testing &lt;br&gt; Therapeutic Intervention &lt;br&gt; Pancreatic enzyme supplementation</td>
</tr>
<tr>
<td></td>
<td>Very Low &lt; 100 mcg/g</td>
<td>Moderate to severe pancreatic insufficiency</td>
<td>Further Testing: &lt;br&gt; Bone Resorption Assessment &lt;br&gt; Glucose/Insulin Analysis &lt;br&gt; Celiac Testing &lt;br&gt; Bacterial Overgrowth of the Small Intestine &lt;br&gt; Therapeutic Interventions &lt;br&gt; Pancreatic enzyme supplementation &lt;br&gt; Vitamin and mineral supplementation</td>
</tr>
<tr>
<td></td>
<td>Normal &gt; 200 mcg/g</td>
<td>Adequate exocrine pancreatic function</td>
<td>No further action necessary. Pancreatic supplementation may be of benefit in low normal (&lt; 400 mcg/g) range</td>
</tr>
</tbody>
</table>

Note: 
<400 Borderline Low
WHO ELSE MIGHT HAVE LOW PE1?

- Celiac disease
- Inflammatory Bowel Disease (IBD)
- HIV
- Diabetes (type 1 and 2)
- Obesity

THE CHALLENGE OF DIGESTION & ABSORPTION

- Breakdown complex foods into basic constituents
- Divide Macronutrients into basic units
- Release Micronutrients from food matrix
- Selectively absorb nutrients
- Transform and/or activate nutrients and phytonutrients
- While maintaining a barrier against entry of unwanted particles
EVERYTHING HAPPENS AT THE INTERFACE!

- Biological systems are designed to create discrete functional units
  - Tissues
  - Cells
  - Organelles
  - Genes

- All of which are equipped to modulate each other by signals at their interfaces

Functional Interfaces require Intact Barriers!
COORDINATED SURVEILLANCE SYSTEMS: PROTECTING “SELF” AT THE INTERFACES

HPA Axis (Stress Response)
- Assessing threats from outside (interface with outside world)
- Compensating for internal imbalances

Immune System
- Surveillance of Self vs. Non-Self
- Highly coordinated by GC signals, highly concentrated in the Gut

Gastrointestinal Tract - GALT
- Maintaining Barrier Function (interface with outside world)
- Signal coordination to brain using direct and immune facilitated signals.
SELYE AND SURVEILLANCE SYSTEM
STRESS

Control  “Stress”

Hypertrophy of Adrenal Gland (HPA)

Atrophy of the thymus and other lymphatic glands (Immune system)

Erosions and ulcers in the duodenum (GI-system)
 INTERFACE OR BARRIER?

“The barrier/permeability functions of the gut represent one of the most important interfaces between a person and the external environment. However, we should not imagine this barrier function as simply a means to keep things out, but as a sophisticated system to communicate with, and allow selective entry of, certain contents from the gut lumen into the body. This requires a tightly controlled, but thin barrier of tissues and secretions intentionally designed for close proximity to the gut lumen. This proximity permits the absorption of available nutrients and physiological interaction with trillions of non-human microbes and their metabolites and signals, but also creates a vulnerability to those same microbes, toxins and immunologically reactive components from the gut lumen.”
EXPANDING THE SURFACE AREA
MORE INTERFACE: MORE VULNERABILITY
THE FUNCTIONAL COMPONENTS OF
THE GUT BARRIER

- Human GI cells that create the interface (Enterocytes, Colonocyte etc.)
- Human Immune cells that line the inside or penetrate the interface
- Human Neuroendocrine cells and neurons with synapses nearby.
- Luminal Excretions from human cells (Mucus, sIgA, anti-microbial peptides, enzymes, acid, neurotransmitters etc.)
- Non-Human microbes in the lumen and mucus lining
  - Commensal, Pathobiont, Pathogenic Bacteria
  - Viruses (free and bacteriophages)
  - Fungi
  - Non-human eukaryotic organisms (are any of these commensals?)
BASIC FEATURES OF THE GUT BARRIER

Small Intestine - Villi and Crypt

Colon - 2 mucus layers, crypt

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STEM CELLS: CONSTANT TURNOVER

**Figure 1: Stem Cell Turnover in the Gut**

- **Crypt-Villus Model:** Stem cells located at the base of the crypts differentiate into various cell types, including Paneth cells, goblet cells, and enterocytes.
- **Cell Shedding:** Cells continuously migrate upward, eventually being shed into the lumen.
- **Mitotic Renewal:** Cells at the base of the crypts undergo mitotic division, replenishing the cells lost to shedding.
- **Differentiation and Migration:** Cells differentiate and migrate upward, becoming more specialized as they move closer to the villus tips.

**Legend:**
- Crypts: Stem cell reservoir
- Paneth cells: Antimicrobial producers
- Goblet cells: Mucus producers
- Enteroendocrine cells: Hormone secretion
- Absorptive epithelial cells: Nutrient absorption
- Proliferative progenitors: Precursors to all cell types

**Key Points:**
- Continuous renewal is crucial for maintaining tissue function.
- The process is tightly regulated to ensure optimal tissue health.

**References:**
- [Expression analysis](#)
- [Regulatory mechanisms](#)
ABSORPTIVE EPITHELIAL CELLS
TIGHT JUNCTIONS

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MLCK- Myosin Light Chain Kinase
ZO-zonula occludens
PANETH CELLS: MANAGING DYSBIOSIS

- Found only in Small Intestines (primarily Ilium)
- Migrate into crypt after differentiation from stem cells
- Secrete antimicrobial peptides (AMPs) into gut lumen
- Are long-lived (months) compared to absorptive cells.
- Help regulate stem cell activity
IMMUNE SYSTEM - TIGHTLY BOUND

Figure 11: Basic Structures of the Gastrointestinal-Associated Lymphoid Tissue (GALT). See the text for detailed explanation.
Most people say “75% of the immune system is in the gut”

This is sort of true, if you only calculate the location of cells. The immune system is the gathering place of naïve B and T cells, and the primary location for peripheral tolerance.

This also requires many innate immune cells, including antigen presenting cells (in the gut: dendritic cells are in control)
SELF-TOLERANCE IS KEY!

**Generative (Primary) Lymphoid Organs**
- Self antigen present in generative lymphoid organ
- Lymphoid precursor
- Newly emerged (immature) clones of lymphocytes
- Maturation of clones not responsive to self antigens present in generative organs

**Central tolerance:** Deletion of lymphocytes specific for self antigens present in generative organs

**Peripheral (Secondary) Lymphoid Tissues**
- Self antigen in peripheral tissues
- Foreign antigen
- Mature lymphocytes
- Immune response to foreign antigens

**Peripheral tolerance:** Deletion or anergy of lymphocytes that recognize self antigens in peripheral tissues
EDUCATION THROUGH SAMPLING NON-SELF FRIEND OR FOE?
DENDRITIC CELLS
PATTERN RECOGNITION RECEPTORS
TOLL-LIKE RECEPTOR FAMILY (TLRS)

Figure: Toll-like receptor (TLR) signaling. This diagram shows the different types of TLRs, their locations and the patterns they recognize. See text for more details about the signaling pathways. Image adapted from Minireview: Toll-like Receptors (TLR)-www.abdserotec.com.
INFLAMMASOME (NLRP3-TYPE)

- Heptamer complex between caspase and NRLP3
- Caspase activates the release of IL-1β, furthering inflammatory cascade
- 3 potential triggers

Cell: Volume 140, Issue 6, 19 March 2010, Pages 821–832
“PARACRINE” FUNCTION OF INFLAMMASOMES IN IBD
INFLAMMASOME AND CHRONIC DISEASE

Type II Diabetes

IL-1β & IL-18

Insulin Resistance & Organ Dysfunction

Pancreas  Adipose Tissue  Liver  Skeletal Muscle  Circulation

NLRP3 inflammasome activation and IL-1β during type 2 diabetes

↓ Insulin secretion  ↑ Inflammation  ↑ Inflammation  ↓ Insulin Sensitivity  ↓ Insulin
↓ Beta cell mass  ↓ Insulin Sensitivity  ↑ Hepatic Steatosis  ↓ Insulin Sensitivity  ↑ Glucose
↑ Beta cell apoptosis  ↓ Insulin Sensitivity

Front. Immunol., 08 March 2013
AUTOINFLAMMATORY DISEASES

Nature Reviews Immunology 12, 570-580 (August 2012)
BASIC FEATURES OF THE COLON BARRIER

- Two layers of Mucus
- Increased number of Goblet Cells
- Less interface, more barrier
- Lower concentration of immune cells
- Fewer Enteroendocrine Cells
- Lumen acts as large fermenting vessel

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LEAKY GUT: MANY DIFFERENT VIEWS

Nature Immunology  14, 685–690 (2013)
“From an MD’s standpoint, it’s a very gray area,” says gastroenterologist Donald Kirby, MD, director of the Center for Human Nutrition at the Cleveland Clinic. “Physicians don’t know enough about the gut, which is our biggest immune system organ.”

"Leaky gut syndrome" isn’t a diagnosis taught in medical school. Instead, "leaky gut really means you’ve got a diagnosis that still needs to be made," Kirby says. “You hope that your doctor is a good-enough Sherlock Holmes, but sometimes it is very hard to make a diagnosis.”

“We don’t know a lot but we know that it exists,” says Linda A. Lee, MD, a gastroenterologist and director of the Johns Hopkins Integrative Medicine and Digestive Center. “In the absence of evidence, we don’t know what it means or what therapies can directly address it.”

From WebMD (page hasn’t been updated since 2013!)
"LEAKY GUT" - MORE COMMONLY USED

**A**

- Diet
- Drugs
- Aging
- Oxidants
- Genetic Predisposition
- Environmental Factors

Microbiota alteration → Dysbiosis → Leaky gut syndrome

**B**

Gut lumen microbiota

- Normal mucosa

Microbiota translocation from lumen to submucosal plexi in the ENS:
  1. Immune priming of EGC through TLRs activation
  2. Enteric neuroinflammation

- Leaky gut

1. Alteration of colon motility
2. Constipation
3. Predromic symptoms of PD

- Prion-like gut-brain transmission
- Inflammation
- Neural death
- SNpc: α-synuclein
- Leaky bodies
- DA loss

- PD symptoms
MORE COMMON SCENARIO
WHAT WE CAN LEARN FROM CELIAC DISEASE

[Diagram: Mechanisms of Disease]

1. Indigestible fragments of gluten induce enterocytes to release the protein zonulin, which loosens tight junctions.

2. Zonulin allows gluten fragments to cross the intestinal lining in abundance and accumulate under epithelial cells (enterocytes).

3. The gluten induces enterocytes to secrete interleukin-15 (IL-15), which activates immune cells called intraepithelial lymphocytes against enterocytes.

4. Antigen-presenting cells of the immune system join the modified gluten to MHC molecules and display the resulting complexes to other immune cells: helper T cells.

5. Helper T cells that recognize the complexes secrete molecules that attract other immune cells and can directly damage enterocytes.

6. B cells release antibody molecules targeted to gluten and TGs. These antibodies might cause further damage when they bind their targets on enterocytes, but their role in the disease is unclear.

7. Killer T cells and natural killer cells can also contribute to the destruction of enterocytes.

8. The enterocytes are destroyed and may be replaced by new cells, leading to the characteristic villus blunting seen in celiac disease.

A. Fasano-Scientific American 301, 54 - 61 (2009)
Mechanisms of gliadin-induced zonulin release, increased intestinal permeability, and onset of autoimmunity.

Zonulin = pre-haptoglobin 2
MEASURING GUT BARRIER FUNCTION

• Gold Standard: Ex-VIVO Ussing Chamber
• Biopsied Tissue (Or experimental monolayer) oriented across membrane
• Can measure Transepithelial electrical resistance (TEER)
• Model system for measuring insults to Gut epithelium
• No support cell structures, no microbiome etc.
MEASURING GUT BARRIER FUNCTION

- In Vivo: size Exclusion Test (Urine Analysis)
  - Lactulose/Mannitol Test most common
  - Mannitol is general measure of gut area, denominator can be altered (low) during atrophy (Celiac, Inflammation etc.)- Ratio can rise even when lactulose levels do not increase due to low mannitol absorption
  - Other test reagents: rhamnose, different size PEG molecules etc.
  - Be careful to follow dietary and timing instructions to prevent false interpretations
OTHER (POTENTIAL) MEASURES OF GUT PERMEABILITY

- Urine/Serum levels of microbial metabolites: D-lactate, endotoxin etc.
- Increased level of bacteria in dense mucus (biopsy)
- Reduced plasma citrulline (biomarker of Glutamine)
- Fecal Calprotectin (Inflammation)
- Measures of TJ proteins [ZO, Claudins, Occludin etc.]
- Serum [or FECAL?] zonulin
GI CONDITIONS FOR WHICH BARRIER FUNCTION IS OFTEN COMPROMISED

- GI Infections (V. Cholera, EH E.coli, C. diff, H. pylori)
- Gut inflammation of any kind likely triggers some gut permeability
- Celiac Disease and 30% of asymptomatic relatives.
- Inflammatory Bowel Disease (both UC and Crohn’s)
- IBS-D (though not stat. sig. in all studies)
- SIBO?
Gut Permeability connected to Obesity, Insulin Resistance and the Western dietary Pattern
Zonulin levels are often increased in obese subjects and type 2 diabetics.


Gut microbiota Richness and Composition and Dietary Intake of Overweight Pregnant Women Are Related to Serum Zonulin Concentration, a Marker for Intestinal Permeability.


Gut microbiota, microinflammation, metabolic profile, and zonulin concentration in obese and normal weight subjects.


Circulating zonulin, a marker of intestinal permeability, is increased in association with obesity-associated insulin resistance.


Serum zonulin is elevated in women with polycystic ovary syndrome and correlates with insulin resistance and severity of anovulation.
THE FUNCTIONAL COMPONENTS OF THE GUT BARRIER

- Human GI cells that create the interface (Enterocytes, Colonocyte etc.)
- Human Immune cells that line the inside or penetrate the interface
- Human Neuroendocrine cells and neurons with synapses nearby.
- Luminal Excretions from human cells (Mucus, sIGA, anti-microbial peptides, enzymes, acid, neurotransmitters etc.)
- Non-Human microbes in the lumen and mucus lining
  - Commensal, Pathobiont, Pathogenic Bacteria
  - Viruses (free and bacteriophages)
  - Fungi
  - Non-human eukaryotic organisms (are any of these commensals?)
IS THIS STILL THE CURRENT STATE OF MICROBIOTA KNOWLEDGE?
• **Commensal Organisms:**
  - the totality of nonpathogenic Organisms that are “natural” residents in or on the host (supplied through the environment or diet).
  - This term can also be used to distinguish these “natural” organisms from “supplemented” organisms that are generally incapable of long-term GI residence (e.g., probiotics).

• **Pathobiont:**
  - This is a commensal organism with the potential for pathogenic activity that, in some circumstances, can trigger negative outcomes for the host (e.g., antibiotics and *C. diff*). These might require the presence of other microorganisms, host immune system dysfunctions or other unknown factors to become pathogenic.
WHAT’S IN YOUR ECOSYSTEM?
WHAT IS THE MOST IMPORTANT TO KNOW?

Most of the information we have is here!

Microbial community

Which microbes are there?
- Nucleic acids
  - SSU rRNA approaches

What are the microbes doing?
- RNA
- Proteins
- Metabolites

What is the genetic potential?
- DNA
- Metagenomics
MICROBIOME DNA ANALYSIS
• Operational Taxonomic Unit (OTU):

• This operational definition of species is used when only genetic material (mostly 16S rRNA) is analyzed to distinguish one species from another. Since many bacteria within the gut microbiome cannot be isolated, grown and investigated in a laboratory setting, they are identified by their genetic sequences and classified into OTUs. Diversity is often described as the number of OTUs. For the clinician, this is functionally identical to the number of species.
DNA TESTING CONUNDRUM

- There is a debate amongst researchers as to the most appropriate measures of metagenetic information to define a person’s or a population’s microbial species.

- Clinically-speaking- we have extremely limited knowledge as to what to do with this information, how to define an ideal microbiome (if such exists) or how to predictably manipulate the microbial environment in a given subject.
Fecal Microbiota Analysis (by any means) is only a biomarker of these microbiomes (heavily skewed to the distal colon)
WHAT IS THE BEST WAY TO DEFINE AN INDIVIDUAL’S GUT MICROBIOME?

• Phylum level Difference?
• Enterotypes?
• Specific OTUs?
• Overall Diversity?
• Presence or absence of specific species?
PHYLUM LEVEL MEASUREMENTS
Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa

Carlotta De Filippo, Duccio Cavalleri, Monica Di Paola, Matteo Ramazzotti, Jean Baptiste Poullet, Sebastien Massart, Silvia Collini, Giuseppe Pieraccini, and Paolo Lionetti

*Department of Pneumology and Clinical Pharmacology, University of Florence, 50134 Firenze, Italy; †Department of Pediatrics, Meyer Children Hospital, University of Florence, 50134 Firenze, Italy; ‡Department of Biochemical Sciences, University of Florence, 50134 Firenze, Italy; §DNA Vision Agrifood S.A., B-4000 Liège, Belgium; and †Centro Interdipartimentale di Spettrometria di Massa, University of Florence, 50139 Firenze, Italy
Human Intestinal Microbiota Composition Is Associated with Local and Systemic Inflammation in Obesity

Froukje J. Verdam,1,2 Susana Fuentes,3 Charlotte de Jonge,1,2 Erwin G. Zoetendal,3 Ruxi Erbil,3 Jan Willem Greve,1,2 Wim A. Buurman,1 Willem M. de Vos3 and Sander S. Rensen1*
COMMERCIAL LABS AND PHYLUM REPORTING
(DOES THIS TELL THE PATIENT ANYTHING?)
Enterotypes of the human gut microbiome

Variations of the human intestinal tract microbiome based on 16S ribosomal RNA encoding gene reported species diversity within and between individuals and the metagenomics studies characterized the functional capabilities of the microbiome of several American and Japanese individuals. Although a general consensus indicates that the phylum-level composition of the human gut is emerging ([8], the variation in species composition and gene pool within the human population is less clear. Furthermore, it is unknown whether there are different human gut microbiome community compositions, as complicated by coexisting, sequencing, enrichment, and core-gene factors, healthy and diseased population, we believe that the global variation of human gut metagenomes.

The vast majority of sequences in the newly sequenced 16S rRNA encoding gene cohort—only 0.9% of the reads could be classified as human contamination, all other reads put together only comprised 0.1%, achieved 90% and exceeds up to 1.0% (see Supplementary Section 2.5 for details).

To investigate the phylogenetic composition of the 16S rRNA encoding gene cohort, we mapped metagenomic reads, using DNA sequence homologs, to the 16S rRNA encoding gene cohort (Supplementary Section 2.5) including 379 publicly available human microbiome genomes generated through the Human Microbiome Project (HMP) (Human Microbiome Project Consortium, 2012) and the MetaHIT project (Heijmans et al., 2012). To consistently estimate the functional composition of the samples, we annotated the predicted genes from the metagenomic data using eggNOG (Kunin et al., 2012) (Supplementary Methods section 6.1). We removed that correspondeds annotating using these protocols not based by data set, or negative annotation, as missing technology and quality filtering (see Supplementary Section 2.6).

"Close" and "far" are the key factors for determining the enterotypes. "Close" and "far" are the key factors for determining the enterotypes. "Close" and "far" are the key factors for determining the enterotypes.
“In light of our findings, we believe that previous analyses produced overconfidence in the claim of discrete enterotypes and that continuous variation is the simpler and therefore better-supported conclusion. Consequently, although discrete clusters may be significantly correlated with a disease state, they may not be appropriate for predicting that disease state due to masking of important within-cluster variation in critical taxa. Finally, in a meta-analysis including both dense single-individual time series data and cross-sectional multiple-individual data, we demonstrated that a healthy adult human's microbiome can traverse much of the total variation space of healthy human gut microbiomes throughout the course of a year, providing evidence that enterotypes are fluid and continuous.”
HOW STABLE IS AN ADULT’S MICROBIOME?

“Nonetheless, overall the set of microbial strains was remarkably stable, with over 70% of the same strains remaining after one year and few additional changes occurring over the following four years. Finally, the stability we document highlights the impact of early colonization events on our microbiota in later life; earlier colonizers, such as those acquired from our parents and siblings, have the potential to provide their metabolic products and exert their immunologic effects for our entire lives.”

From: Guilliams TG. Functional Strategies for the Management of Gastrointestinal Disorders (Point Institute, 2016)
SOME PRECAUTIONS

- We don’t yet know what is “Normal”
- Most commercial Lab Data is not Reproducible enough to adequately deciphering Stool samples for diversity
- Stool samples may be representative of some portions, but perhaps not the most important features of the microbiome
FUNCTION MATTERS

- Altered patterns within the microbiota in early life may influence health long after those patterns are discernable through microbiome testing (Know the patient’s History!)

Nature 489, 220–230 (13 September 2012) doi:10.1038/nature11550
FUNCTION MATTERS

• The presence or absence of specific species of (or genes from) bacteria may be less important than the Gene expression, Proteome or Metabolome in the Gut.
THE BASIC ROLES OF GI MICROBIOTA

Protection
- Niche competition
- Pathogen displacement
- Nutrient competition
- Receptor competition
- Production of anti-microbial factors (e.g., bacteriocins, lactic acids)

Structural
- Barrier fortification
- Induction of IgA
- Apical tightening of tight junctions
- Immune system development and maintenance

Metabolic and Signaling
- Control of epithelial cell differentiation and proliferation
- Metabolize dietary carcinogens
- Synthesize vitamins (e.g., vitamin K, biotin, folate, etc.) neurotransmitters, amino acids, short chain fatty acids (e.g., butyrate, propionate, acetate)
- Ferment non-digestible dietary components and epithelial derived mucus
- Ion and mineral absorption
- Salvage energy
- Detoxification and biotransformation of hormones, toxins, medications, bile acids, phytosteroids
GUT MICROBIOME AND CVD

From: Guilliams TG: Cardiometabolic Risk Management- A Functional and Lifestyle Approach (Point Institute 2018)
THE POWER OF ADAPTABILITY

- 50-150 billion cells
- Fixed Genome, slowly change epigenome
- Influenced by genomic signals

- ~1 Trillion Cells
- 30-40% altered genome in few days
- Influenced by genomic signals
- Epigenome?
MICROBIOME/IMMUNE INTERFACE
COMMENSAL FLORA AND IMMUNE CONTROL
IMMEDIATE FACTORS THAT INFLUENCE FECAL MICROBIOME SAMPLES.

- Stool Morphology as measured by Bristol Stool Scale
  - Transit Time
  - Available time for fermentation/division
  - Individuals with a short transit time may have greater amounts of fast-growing bacterial species, while those with slow transit times may instead select bacteria with greater adherence to host tissue.

- The Use of Medications
  - Antibiotics
  - Laxative
  - PPIs

THE BIGGEST LEVERS TO CHANGE THE HUMAN GUT MICROBIOME

- Diverse Diet, variety of plants
  - Source of New Microbes
  - Food for Existing Microbes
- Fecal Microbial Transplants (short-term?)
- Probiotics (short-term)

- Processed Food Diet
- Broad spectrum Antibiotics
Here we show that the short-term consumption of diets composed entirely of animal or plant products alters microbial community structure and overwhelms inter-individual differences in microbial gene expression. Microbial activity mirrored differences between herbivorous and carnivorous mammals, reflecting trade-offs between carbohydrate and protein fermentation. Foodborne microbes from both diets transiently colonized the gut, including bacteria, fungi and even viruses. In concert, these results demonstrate that the gut microbiome can rapidly respond to altered diet, potentially facilitating the diversity of human dietary lifestyles. Nature. 2014 Jan 23;505(7484):559-63.
THE HEAVY HAND OF ANTIBIOTIC THERAPY

Antibiotics
- Depletion of bacterial diversity
- Altered gene expression, protein activity and overall metabolism
- Selection for intrinsically resistant bacteria
- Selection for new mutations and gene transfers conferring resistance

Increased Susceptibility to Infections by Exogenous Pathogens or Opportunistic Commensals
- Loss of potential competitors
- Lower expression of antibacterials and IgG
- Decrease in neutrophil-mediated killing

Compromised Immune Homeostasis
- Disruption of Treg/Th balance
- Elevated inflammatory signals
- Related to atopic, inflammatory and autoimmune diseases (allergies, asthma, necrotizing enterocolitis, inflammatory bowel disease, irritable bowel syndrome, etc.)

Dysregulated Metabolism
- Elevated inflammatory signals
- Altered insulin sensitivity
- Altered metabolism of SCFA and bile acids
- Related to obesity, metabolic syndrome, diabetes

Accumulation of Antibiotic Resistances
- Establishment of resistant bacteria
- Transfer of resistance genes to pathogens
- May result in untreatable bacterial infections

© Guilliams: GI Roadmap- Point Institute 2016
• How much of the past 100 year shift in human metabolic function might have been caused by a massive shift in our microbiome due to the rampant use of antibiotics?

Phylum-level changes in animals given antibiotics
Failure Rate (need for new antibiotic within 2-18 days of first prescription) was ~10%
C. DIFF AND ANTIBIOTICS

- Taking antibiotics is the top risk factor for developing *C. difficile* infections for both children and adults.

- Researchers found that 71 percent of cases of *Clostridium difficile* infection among American children aged 1 to 17 occurred shortly after they took antibiotics that were prescribed in doctors' offices to treat other conditions.

- Most of the children received antibiotics for problems such as ear, sinus or upper respiratory infections. Previous research has shown that at least 50 percent of antibiotics prescribed to children in doctors' offices are for respiratory infections, most of which do not require antibiotics.

U.S. Centers for Disease Control and Prevention, news release, March 7, 2014
BIOFILM: A CLASSIC APPROACH, BUT NOT LIKELY A MODEL FOR GI ORGANISMS
Excellent Review of biofilm

Reveals we still know very little about biofilm’s beneficial and detrimental functions – and what we can do about it.

Beneficial activities potentially attributed to some exopolysaccharides synthesized by Bifidobacterium.

THE MUCOSAL MICRO-ENVIRONMENTS
MUCUS FACTORS THAT INFLUENCE MICROENVIRONMENTS

B. Mucus Rigidity
C. Fluid Shear Gradients
D. Oxygen Gradients
E. Host Defense Molecules
F. Mucosal Nutrient Platform
G. Crypt Niche
Colonic Microbiota Encroachment Correlates With Dysglycemia in Humans

Benoit Chassaing, Shreya M. Raja, James D. Lewis, Shanthi Srinivasan, and Andrew T. Gewirtz

1Center for Inflammation, Immunity and Infection, Institute for Biomedical Sciences, Georgia State University, Atlanta, Georgia; 2Digestive Diseases Division, Department of Medicine, Emory University School of Medicine, Atlanta, Georgia; 3Atlanta VA Medical Center, Decatur, Georgia; 4Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania
Several natural agents (and synthetic analogs) are known to disturb biofilm formation or inhibit quorum sensing in laboratory tests, but we are not aware of any research specific to mucosal biofilms generally, or gastrointestinal outcomes specifically.

We are very cautious on recommending GI “biofilm-disrupting” therapies because biofilm communities within the GI mucosa contain heterogeneous mixtures of mostly beneficial organisms along with those that are potentially harmful.

Biofilm disruption is unlikely to discriminate between the good and the bad.

Furthermore, virtually no evidence exists to guide the clinician in the selection of agents, doses and length of treatment for GI biofilm disrupting therapies related to GI-related clinical outcomes.

The use of ingredients that perform well in in vitro tests of biofilm disruption of monocultures, have yet to be shown effective or beneficial for GI-related outcomes (though some are marketed as if they have).
THE MICROBIOME AND PHYTOTHERAPY

© Guilliams: GI Roadmap- Point Institute 2016
BERBERINE- A COMPREHENSIVE METABOLIC SIGNALING MOLECULE.

An isoquinoline alkaloid found in plants like *Berberis aquifolium* (Oregon grape), *Berberis vulgaris* (barberry), *Berberis aristata* (tree turmeric), *Hydrastis canadensis* (goldenseal), *Xanthorrhiza simplicissima* (yellowroot) and *Coptis chinensis* (Chinese goldthread)- the common source for commercial berberine HCL/Sulfate.
Berberine is a novel cholesterol-lowering drug working through a unique mechanism distinct from statins

Weina Kong1, Jing Wei2, Parvaneh Afshari1, Mei Hong Li1, Sitabara samak3, Cong Li5, Fei Wang5, Zhipeng Wang6, Yan Li2, Shunfu Wang5, Menglan Wei5, Yang Wang5, Zhihong Li1, Jingwen Liu1, Jingwen Liu1

We identify berberine (BBR), a compound isolated from a Chinese herb, as a new cholesterol-lowering drug. Oral administration of BBR in 12 hypercholesterolemic patients for 4 weeks reduced serum cholesterol by 29%, triglycerides by 23% and LDL-cholesterol by 34%. Treatment of hypercholesterolemic mice with BBR reduced serum cholesterol by 40% and LDL-cholesterol by 48%, with a 3.8-fold increase in hepatic LDLR mRNA and a 3.5-fold increase in hepatic LDLR protein. When human hepatoma cells were co-cultured with BBR, we found that BBR increased LDLR expression independent of the regulatory element binding protein, but dependent on HRE activation. BBR elevates LDLR expression through a post-transcriptional mechanism that stabilizes the HRE. Using a heterozygous mouse with a deletion as a reporter, we further identify the HRE proximal section of the LDLR promoter 3′ untranslated region responsive for the regulatory effect of BBR. These findings show BBR as a new hypolipidemic drug with a mechanism of action different from that of statin drugs.

The expression of the low-density lipoprotein receptor (LDLR) regulates plasma lipoprotein levels, and serum LDL-C concentrations. As BBR can increase LDLR expression in hepatic cells, it may promote the clearance of plasma LDL-C through receptor-mediated mechanisms, which have been strongly associated with a decreased risk of developing cardiovascular disease in humans2,3. LDLR expression is predominantly regulated at the transcriptional level through a negative feedback mechanism by the low density lipoprotein receptor (LDLR) promoter. This regulation is controlled through specific interactions of intracellular regulatory elements (HREs) and the low density lipoprotein receptor (LDLR) promoter, which control transcriptional activity in the promoter element responsive to the (LDLR) promoter. In this study, we explore the transcriptional regulation of the LDLR gene, which is controlled by the LDLR promoter and the LDLR gene expression. Under cholesterol-saturated conditions, LDLR expression is increased in vivo and in vitro in the presence of specific transcription factors and transcription factors. The LDLR gene expression is maintained at a minimum mRNA level, as indicated by steady-state mRNA levels. Under cholesterol-saturated conditions, LDLR expression is increased in vivo and in vitro in the presence of specific transcription factors and transcription factors.

Clinical studies have been the most suitable and excellent examples for hypercholesterolemia. In this study, we explored the regulatory mechanisms of BBR on the LDLR gene and examined whether BBR can increase LDLR expression in vivo and in vitro. We found that BBR increased LDLR expression through a post-transcriptional mechanism that stabilizes the HRE. Using a heterozygous mouse with a deletion as a reporter, we further identified the HRE proximal section of the LDLR promoter 3′ untranslated region responsive for the regulatory effect of BBR. These findings show BBR as a new hypolipidemic drug with a mechanism of action different from that of statin drugs.

**Table 1: Effects of BBR on serum lipids in the subgroup of hypercholesterolemic patients**

<table>
<thead>
<tr>
<th>Treatment (3 months)</th>
<th>BBR</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum level of cholesterol</td>
<td>(&gt;5.2 mmol/L, n = 32)</td>
<td>(&gt;5.2 mmol/L, n = 11)</td>
</tr>
<tr>
<td>Cholesterol Before</td>
<td>5.9 ± 0.7</td>
<td>6.1 ± 0.6</td>
</tr>
<tr>
<td>After</td>
<td>4.2 ± 0.7</td>
<td>6.0 ± 0.8</td>
</tr>
<tr>
<td>Triglyceride Before</td>
<td>2.3 ± 1.0</td>
<td>2.2 ± 0.8</td>
</tr>
<tr>
<td>After</td>
<td>1.5 ± 0.7</td>
<td>2.1 ± 0.9</td>
</tr>
<tr>
<td>HDL-c Before</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.5</td>
</tr>
<tr>
<td>After</td>
<td>1.1 ± 0.3</td>
<td>1.2 ± 0.4</td>
</tr>
<tr>
<td>LDL-c Before</td>
<td>3.2 ± 0.7</td>
<td>3.7 ± 0.7</td>
</tr>
<tr>
<td>After</td>
<td>2.4 ± 0.6</td>
<td>3.7 ± 0.8</td>
</tr>
</tbody>
</table>

*Statistical analysis of the baseline of cholesterol, triglyceride, HDL-c and LDL-c showed that there were no significant differences between the BBR and placebo groups before therapy (P > 0.05), ***P < 0.001, as compared to the baseline of before treatment group.*

**Table 2: Effect of BBR on liver and kidney functions of the subgroup of hypercholesterolemic patients**

<table>
<thead>
<tr>
<th>Group</th>
<th>ALT (U/L)</th>
<th>AST (U/L)</th>
<th>GGT (U/L)</th>
<th>ALP (U/L)</th>
<th>BUN (mmol/L)</th>
<th>Cr (mmol/L)</th>
<th>Na (mmol/L)</th>
<th>K (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before treatment</td>
<td>26 ± 11</td>
<td>34 ± 15</td>
<td>45 ± 12</td>
<td>23 ± 7</td>
<td>54 ± 12</td>
<td>25 ± 5</td>
<td>140 ± 10</td>
<td>3.5 ± 0.5</td>
</tr>
<tr>
<td>After treatment</td>
<td>23 ± 10</td>
<td>30 ± 10</td>
<td>40 ± 10</td>
<td>22 ± 7</td>
<td>50 ± 10</td>
<td>24 ± 5</td>
<td>140 ± 10</td>
<td>3.4 ± 0.5</td>
</tr>
</tbody>
</table>

**Standard deviation of the baseline of liver and kidney functions of the BBR and placebo groups before therapy (P > 0.05), ***P < 0.001, as compared to the baseline of before treatment group.**

---

1. Institute of Medical Genetics, Chinese Academy of Medical Sciences, and Peking Union Medical College, Beijing, China
2. Department of Cardiology, Beijing Medical University, Beijing, China
3. Department of Cardiology, Beijing Medical University, Beijing, China
4. Department of Cardiology, Beijing Medical University, Beijing, China

5. National Clinical Laboratory Manual issued by The Ministry of Health of the People’s Republic of China, with minor modifications, *P < 0.01, **P < 0.001, as compared to those before treatment. ALP, alkaline phosphatase; AST, aspartate transaminase; ALT, alanine transaminase; GGT, γ-glutamyl transpeptidase; BUN, blood urea nitrogen; Na, sodium; K, potassium.

6. Standard deviation of the baseline of liver and kidney functions of the BBR and placebo groups before therapy (P > 0.05), ***P < 0.001, as compared to the baseline of before treatment group.
Berberine lowers blood glucose in type 2 diabetes mellitus patients through increasing insulin receptor expression

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Abstract

Our previous work demonstrated that berberine (BBR) increased insulin receptor (IR) expression and improved glucose uptake in vitro and in animal models. Here, we studied the cell-sensing ability and glucose-lowering activity of BBR in humans. Our results showed that BBR increased insulin receptor messenger RNA and protein expression in a variety of human cell lines, including C2C12, HUT, H9C2, and HepG2. In keratinocytes, BBR also increased insulin receptor expression. In the clinical study, BBR significantly lowered fasting blood glucose (FBG), hemoglobin A1c (HbA1c), and the levels of fasting FBG and hemoglobin A1c (HbA1c) in response to a meal. The glucose-lowering effect of BBR was similar in those with normal and impaired glucose tolerance. In the BBR-treated group, the percentage of patients with normal FBG and hemooglobin A1c was significantly increased. Berberine also lowered FBG effectively in ethanol-dependent and severe poor compliance patients with type 2 diabetes mellitus (T2DM). Together with our previous work, we propose that BBR is an ideal candidate for T2DM with a mechanism different from metformin and sulfonylurea.

1. Introduction

The insulin receptor (IR) is a transmembrane glycoprotein that is essential for the action of insulin. Binding of insulin to IR in the liver, muscles, or adipose tissue triggers multiple intracellular pathways that cause glycolysis, glycogen synthesis, and glucose uptake. The insulin receptor is thus a critical regulator of glucose homeostasis.

Table 1: Effects of BBR, metformin, and rosiglitazone in T2DM patients

<table>
<thead>
<tr>
<th>Table 1: Effects of BBR, metformin, and rosiglitazone in T2DM patients</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Efficacy (mg/ml)</strong></td>
</tr>
<tr>
<td><strong>Before</strong></td>
</tr>
<tr>
<td>FBG (mmol/L)</td>
</tr>
<tr>
<td>HbA1c (%)</td>
</tr>
<tr>
<td>TG (mmol/L)</td>
</tr>
</tbody>
</table>

Values are mean ± SEM. *P < 0.01 compared with before treatment by paired t-test.

1 gram BBR per day
EXCELLENT REVIEW OF BERBERINE’S MECHANISMS AND CLINICAL RESULTS

SIGNALING PATHWAYS TRIGGERED BY BERBERINE
BERBERINE AND THE GUT MICROBIOME
 HOW THEY AFFECT EACH OTHER.

• One the one hand: Berberine appears to affect physiology, partly by modulating gut microbiome.
Taken together, our findings suggest that the prevention of obesity and insulin resistance by berberine in HFD-fed rats is at least partially mediated by structural modulation of the gut microbiota, which may help to alleviate inflammation by reducing the exogenous antigen load in the host and elevating SCFA levels in the intestine.
ANTIBIOTICS INHIBIT THE BENEFIT AND BIOAVAILABILITY OF BERBERINE IN ANIMAL MODELS

Change in FBG

Change in TG

Transforming berberine into its intestine-absorbable form by the gut microbiota.


However, oral doses of dhBBR are unstable, and convert back to BBR before reaching the gut.
Significant pharmacokinetic differences of berberine are attributable to variations in gut microbiota between Africans and Chinese

Plasma levels of Berberine
HOW MANY PHYTOCHEMICALS NEED A HEALTHY MICROBIOTA FOR ACTIVATION?
In many cases “microbiota availability” may actually be the target of phytochemicals that are known to have poor human bioavailability.

Our desire to increase bioavailability of important phytochemicals may actually miss the very target of their therapy, or at least alter that relationship substantially.
• FMT are now recognized globally as highly successful for recurrent *C. difficile* infections (CDI/CDAD)
• In appropriate subjects (with appropriate donors), successful remission is >90% (Fresh or Frozen!)
• Success in children for CDI is similar, though fewer studies have been performed. *Pediatr Res.* 2016 Jul;80(1):2-6.
• FMT studies on other GI conditions (IBD, IBS, etc.) and non-GI conditions: obesity, immune-related outcomes, autism etc. are ongoing with some success in small trials. *Dig Dis Sci.* 2017 May;62(5):1131-1145.
WHAT ABOUT PROBIOTICS?

Stay Tuned.....
• Most of what we know is good (or Bad) for our microbiome(s) has already been shown to be good (or Bad) for Us- With few exceptions.

• the Microbiome revolution helps explain how and why certain lifestyle interventions may work, but rarely contradicts what we know about healthy diets, physical activity, stress, Hygiene etc.
CHRONIC DISEASE MANAGEMENT REQUIRES SUPPORTING THE CORE FUNCTIONS OF GI

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CORE FUNCTIONS VS 4R (OR 5R)

**REMOVE** *(Important First Step in 4R Model)*
Promote Elimination and Detoxification
- Remove Allergens and Toxins
  - Elimination diet
  - Detoxification protocol
- Remove Harmful Organisms
  - Stool testing for pathogens
  - Eliminate pathogens

**REPLACE**
Promote Digestion and Absorption
- Supplement or stimulate
  - Stomach acid
  - Digestive enzymes
  - Bile for fat absorption
  - Easy to absorb nutrients

**RE-ESTABLISH** *(Re-inoculate)*
Ecosystem for Microbiome
- Microbiome-friendly diet
- Avoiding certain drugs/antibiotics
- Probiotics
- Prebiotics

**REPAIR**
Barrier Function/Immune Interface
- Reduce gut inflammation
- Provide nutrients for GI cells
- Improve tight junctions
- Increase signals for immune modulation

**SUPPORTING NEUROENDOCRINE (GUT/BRAIN) FUNCTION**
- Modulate the effects of HPA axis/stress
- Control neurotransmitter synthesis and function
- Manage satiety signals from gut
- Coordinate signals from microbiome, immune system, bowel transit to and from the CNS

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