Supporting Gut Barrier Function

Thomas G. Guilliams, PhD
Point Institute- Stevens Point, WI (USA)

September 27th, 2017

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Core Functions of the GI
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.

Everything Happens at the Interface!
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 vs. "Stress"

- Hypertrophy of Adrenal Gland (HPA)
- Atrophy of the thymus and other lymphatic glands (Immune system)
- Erosions and ulcers in the duodenum (GI-system)
“Therefore, 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
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**
Stem Cells: Constant Turnover
Absorptive Epithelial Cells

Tight Junctions

MLCK - myosin light chain kinase
ZO - zonula occludens
Digestion and Absorption

Transcellular transport of key nutrients coordinated by enzymes and transporters within absorptive cells in small intestine
Paneth Cells: Managing Dysbiosis

- Found only in small intestine (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
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
The Mucosal Micro-Environments
Mucus Factors That Influence Microenvironments

I. Mucus rigidity
II. Fluid shear gradients
III. Oxygen gradients
IV. Host defence molecules
V. Mucosal nutrient platform
VI. Crypt niche
Is “Leaky Gut” a Legitimate Term?

• “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.” - WebMD

"Leaky Gut" on PubMed
"Leaky gut" - More Commonly Used
Leaky Gut: An Extreme View

More Common Scenario
What We Can Learn From Celiac Disease
Mechanisms of Gliadin Induced Zonulin Release, Increased Intestinal Permeability, and Onset of Autoimmunity
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, occluding, etc.)
- Serum (or fecal) zonulin
Conditions for Which Barrier Function is Compromised

• GI Infections (V.cholera, EH E.coli, C.diff, H.pylori, etc...)
• 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 Disease)
• IBS-D (though not statistically significant in all studies)
• SIBO?
Gut Permeability Connected to Obesity, Insulin Resistance and the Western Dietary Pattern

Zonulin Levels are Increased in Obese Subjects and Type 2 Diabetics

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


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


**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.**

Preventing and Treating Intestinal Permeability

• Since intestinal permeability is rarely a “stand-alone” diagnostic criteria, there are few studies that investigate this as a primary outcome
• Many studies looking at in-vitro changes in barrier function (Ussing Chamber) and animal studies looking for influences of nutrients
• Few human clinical trials so far
Dietary Interventions

- It is assumed that common dietary patterns that induce poor microbiome balance, inflammation and chronic disease (excessive fat, refined carbohydrates, phytonutrient poor) are linked with increased intestinal permeability.
- Few studies using dietary interventions have looked at changes in gut permeability.
Inulin-enriched pasta improves intestinal permeability and modifies the circulating levels of zonulin and glucagon-like peptide 2 in healthy young volunteers

- 5 week crossover design with 8-week washout
- Healthy young (mean age 18.8 years old)
- 100 grams pasta/day
- Wheat pasta with or without 11% inulin (prebiotic from chicory)
Micronutrients and Barrier Function

- Compromised barrier function is related to deficiencies in:
  - Vitamin A / beta carotene
  - Vitamin D
  - Zinc
  - Iron

- Intervention studies are limited to vitamin A + zinc supplementation in malnourished children (improved barrier function) and zinc in IBD and exercise induced permeability

L-Glutamine and Barrier Function

- Long list of mechanisms
- Numerous animal models to show GI barrier benefits
- Most human data related to severe injury/burn victims
- Frequent use within integrative/functional medicine community (anecdotal success)
- Recent limited research in traditional gut barrier human clinical research
Some mechanisms linking glutamine with intestinal barrier functions:

• Needed for the development of the gut epithelium during early life supplementation (used in neonates to improve gut barrier function)

• Critical substrate for metabolites within enterocytes including ATP, glutathione and DNA/RNA

• Important secondary signaling molecule within enterocytes, affecting critical metabolic and proliferative pathways in the cell

• GLN has been shown to modulate TJ proteins, phosphorylation and assembly, using both GLN deprivation and supplementation studies

• GLN contributes to favorable alterations in the gut microbiota

• GLN maintains intestinal structure and function during aging

• GLN promotes sIgA secretion via direct (immunomodulatory) and indirect (microbiota) signals

• GLN modulates the GI permeability effects of HPA axis stress (i.e., CRF)

• GLN modulates the gastrointestinal permeability effects of intensive exercise
The Roles of Glutamine in the Intestine and Its Implication in Intestinal Diseases

Protection against apoptosis and cellular stresses

Heat shock proteins
(HSP-70, HSP-25, HSP-72)

Autoxidation
ER stress
mTOR
MAPKs
(ERK1/2, JNK)

Growth factors
(EGF, IGF-1, TGF-α)

Glutamine

NF-κB
GSH
STAT-1, STAT-3,
STAT-4, STAT-5

Tight junctions
(Claudin-1, Claudin-4,
occludin, ZO-1, ZO-2, ZO-3)

Anti-
inflammation

Maintaining intestinal tissue integrity

GLN Studies in Undernourished Children or Severe Illness

Limited data on “functional” GI situation

Effects of glutamine alone or in combination with zinc and vitamin A on growth, intestinal barrier function, stress and satiety-related hormones in Brazilian shantytown children


Original Article

Efficacy of glutamine-enriched enteral feeding formulae in critically ill patients: a systematic review and meta-analysis of randomized controlled trials

Azadeh Mottaghi PhD, Maryam Zaref Yeganeh MSc, Mahdieh Golzarand MSc, Sara Jambarsang MSc, Parvin Mirmiran PhD


Glutamine and Whey Protein Improve Intestinal Permeability and Morphology in Patients with Crohn’s Disease: A Randomized Controlled Trial

Jaya Benjamin · Govind Makharia · Vinod Ahuja · K. D. Anand Rajan · Mani Kalaiyan · Siddhartha Datta Gupta · Yogendra Kumar Joshi

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Abstract
Background Increased intestinal permeability (IP) has been implicated in the etiopathogenesis, disease activity and relapse of Crohn’s disease (CD). Glutamine, the major fuel for the enterocytes, may improve IP.

Aim We evaluated the effect of oral glutamine on IP and intestinal morphology in patients with CD.

Methods In a randomized controlled trial, consecutive patients with CD in remission phase with an abnormal IP were randomized to a glutamine group (GG) or active control group (ACG) and were given oral glutamine or whey protein, respectively, as 0.5 g/kg ideal body weight/day for 2 months. IP was assessed by the lactulose mannitol excretion ratio (LMR) in urine, and morphology was performed by computerized image analysis system.

Results Patients (age 34.5 ± 10.5 years; 20 males) were assigned to the GG (n = 15) or ACG (n = 15). Eighteen patients in each group completed the trial. (median (range)) in GG and ACG at 2 months were 0.006 (0.00-0.090) and 0.033 (0.009-0.077), respectively, with P = 0.0133. IP normalized in 8 (57.1%) patients in each group (P = 1.00). The villous crypt ratio (VCR) (mean (SD)) in GG and ACG at 2 months was 2.68 (1.02) and 2.49 (0.67), respectively, (P = 0.347). At the end of 2 months LMR improved significantly in GG from 0.071 (0.01-0.254) to 0.029 (0.006-0.090) (P = 0.0012) and in ACG from 0.067 (0.040-0.136) to 0.033 (0.009-0.077) (P = 0.0063). VCR improved in the GG from 2.33 (0.77) to 2.68 (1.02) (P = 0.001) and in ACG from 2.32 (0.57) to 2.49 (0.67) (P = 0.009).

Conclusions Intestinal permeability and morphology improved significantly in both glutamine and ACG.

40 grams of glutamine (80kg-176 lbs)
Glutamine Supplementation and Immune Function During Heavy Load Training

- 24 athletes given 10 gram/day GLN or placebo for 6 weeks
- GLN was able to attenuate immunosuppression triggered by intense heavy-load training compared to placebo
- Gut permeability was not assessed in these subjects
Glutamine for Gut Support

• One of the most common recommendations for supporting gut barrier, though with limited published support (considered to be very safe for nearly all subjects)
• Dose recommendation starts at 4 to 8 grams/day, but may need much higher doses for desired outcome
• Often provided in powder rather than capsules to allow for higher dose therapies
Flavonoids (of all kinds) have been shown to promote strong tight junction formation when tested in vitro (directly signaling enterocytes).

Flavonoids have been shown to create a diverse and healthy gut population (indirect benefit on barrier).

Many supplemental flavonoids are potent anti-inflammatory agents.

Diverse diet is best option, followed by range of flavonoids via supplementation (dose not as important as long-term use).
Berberine

• Popular alkaloid for antimicrobial and metabolic-related outcomes (LDL-C, FBG, Met-syn, BP, etc.)
• Numerous in vitro and animal studies suggesting potent affects on improving TJ formation and function
• Berberine has affect on microbiome and type 2 diabetes, both known to affect or be affected by gut barrier issues

Berberine Attenuates Intestinal Mucosal Barrier Dysfunction in Type 2 Diabetic Rats

A Randomized Clinical Trial of Berberine Hydrochloride in Patients with Diarrhea-Predominant Irritable Bowel Syndrome

400 mg/day berberine

Did not measure gut permeability in IBS-D subjects
Probiotics

- A wide-range of probiotics are likely to help balance the microbiome, benefit immune function and improve gut barrier function...however
- Few clinical trials actually measure gut barrier function as an outcome in probiotic clinical trials
Many In-Vitro Studies


**Strengthening of the intestinal epithelial tight junction by Bifidobacterium bifidum.**
Heath SJ*, Osawa T*, Moriyama E*, Datta Y*, Aikata H*, Tsuchida S.*


**Protective effects of Lactobacillus plantarum on epithelial barrier disruption caused by enterotoxigenic Escherichia coli in intestinal porcine epithelial cells.**

Influenza Res. 2016 Dec;22(12):2811-2823.

**VSL#3 Probiotic Stimulates T-cell Protein Tyrosine Phosphatase-mediated Recovery of IFN-γ-induced Intestinal Epithelial Barrier Defects.**
Keshavan M*, Pennero HM, Shah NN, Marchetti BR, McCole DF.


**Secretions of Bifidobacterium infantis and Lactobacillus acidophilus Protect Intestinal Epithelial Barrier Function.**

J Clin Invest. 2017 Feb 22. doi: 10.1172/JCI93843 [Epub ahead of print]

**Saccharomyces boulardii CNCM I-745 restores intestinal barrier integrity by regulation of E-cadherin recycling.**
Most studies published in Chinese (poor quality-low JADAD score)
Most probiotics showed improvement in variety of TJ, inflammatory markers and L/M when measured
Large heterogeneity of probiotic strains and doses
• 160 Subjects given probiotic or placebo for 6 days prior to surgery and 10 days after

• The probiotic was delivered in acid-resistant capsules containing 2 g of a mixture of *L. plantarum* (>10^{11} CFU/gram), *L. acidophilus* (>7x10^{10} CFU/gram) and *B. longum* (>5x10^{10} CFU/gram)

• The postoperative serum zonulin concentration in the control group (1.08 ± 0.28 ng/mg protein) was significantly higher than that in the probiotics group (0.39 ± 0.26 ng/mg protein; P = 0.001

Fecal zonulin decreased with supplementation from values slightly above normal into normal ranges (<30 ng/ml) and was significantly lower after 14 weeks with probiotics compared to placebo ($p = 0.019$). At baseline, both groups showed considerably higher TNF-α concentrations than normal. After 14 weeks TNF-α was tendentially lower in the supplemented group ($p = 0.054$). IL-6 increased significantly from pre to post exercise in both groups ($p = 0.001$), but supplementation had no effect.

**Conclusions** The probiotic treatment decreased zonulin in feces, a marker indicating enhanced gut permeability. Moreover, probiotic supplementation beneficially affected TNF-α and exercise induced protein oxidation. These results demonstrate promising benefits for probiotic use in trained men.
• 34 Crohn’s patients randomized for treatment with either placebo or Saccharomyces boulardii. Baseline medications (mesalamine, azathioprine, prednisone, metronidazole and/or thalidomide) were maintained
• 400 million CFU of S. boulardii every eight hours
• Intestinal permeability (lactulose/mannitol ratio) was evaluated immediately before the beginning of treatment and at the end of the first and third treatment month
• Fifteen healthy volunteers were also submitted for the intestinal permeability test
16 Athletes given either placebo (whey) or 500 mg of bovine colostrum (both with 500 mg of desiccated banana) for 20 days

- Tested during peak training when it is known that gut permeability is compromised
• Discover and avoid foods known to cause increased intestinal permeability
  – May include: gluten, dairy/lactose, capsicum/spicy foods, FODMAPs, etc.
  – Test for (and avoid) food allergens (IgE/mast cell stimulation)
• Cease NSAID use if possible
• Assess HPA axis stressors and treat accordingly - stress directly influences gut permeability
• Avoid strenuous physical activity/exercise or pay special attention to supporting gut and immune health before and after such activities - moderate exercise is helpful
• Avoid processed foods with artificial colors and flavors
• Eat abundant amounts of fresh fruits and vegetables to maximize the amount and diversity of phytonutrients
Consider the following nutrients for supplementation:

- Omega-3 fatty acids, ALA, EPA, DHA (through diet and supplementation)
- Glutamine (4 to 8 grams daily)
- Vitamin D (1,000 IU minimum daily; best to test and dose to desired serum levels)
- Probiotics (mixed strain combination 20-40 billion CFU; consider high doses for long-standing intestinal barrier issues or when associated with IBD)
- Prebiotics (precursor for important short-chain fatty acids - may be contraindicated if FODMAPs are to be avoided)
- Zinc (25 mg daily with other minerals)
- Iron (only when iron deficiency is confirmed)
- Flavonoids (for quercetin and related compounds, dose not as important as consistent daily consumption from foods and supplementation)
- Colostrum/Lactoferrin/IgG
- Berberine (consider adding 1 g/day when subject is obese, insulin-resistant or has type 2 diabetes)
Questions?

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www.pointinstitute.org – (For Book Purchases and White Papers)
Please schedule a complimentary appointment with one of our Medical Education Specialists for questions related to:

- Diagnostic profiles featured in this webinar
- How Genova’s profiles might support patients in your clinical practice
- Review a profile that has already been completed on one of your patients

We look forward to hearing from you!
Genova Events Update

• MICROBIOME SYMPOSIUM – WASHINGTON, DC
  – A full day event with Dr. Todd LePine
    • October 28th, 2017
    • Register @ attend.gxd.net
  
  – Topics included:
    • Learn how gut bacteria impacts health and disease
    • Learn how impaired digestion and absorption can affect GI bacteria and nutrient status
    • Discover the importance of GI barrier function and the immune system reactions...and more!
Upcoming LIVE GDX Webinar Topics

October 25, 2017 – Dr. Doreen Saltiel Presents:

Advancing Patient Management:
The Role of Genetics in Cardiovascular Disease

Register for upcoming LIVE GDX Webinars online at WWW.GDX.NET

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