The Microbiome in Eye & Systemic Disease: What You Need to Know

A. Paul Chous, MA, OD, FAAO
Tacoma, WA

Disclosures

- I have been a consultant for, been on advisory boards or spoken on behalf of the following:
  - AI Optics, Bausch & Lomb, EyeNuk, Genentech, Glaxo Smith Kline, Novo Nordisk, Prodigy Diabetes Care, Regeneron, Risk Medical Solutions, VSP, Zeavision, Zeiss

What is our microbiome

- 10–100 trillion symbiotic microbial cells harbored by each person, primarily bacteria in the gut – but also skin, mucus membranes and the ocular surface
- Microbiome also houses viral strains, yeasts, fungi, protozoa and other single-celled prokaryotes
- Influenced by multiple external/environmental factors & affects/controls physiological processes (AKA “Our Second Brain”)

Enteric Nervous System

- 100 million neurons in the gut wall
- More than the spinal cord
- 90% of vagus nerve fibers carry information from the gut to the brain, NOT vice versa
- 90% of all serotonin produced in the gut

Cell. 2015 Apr;161(2):264-76.
You are mostly not you!

- Approx 100 TRILLION bacterial cells vs 30 Trillion "Human" cells in you
- Approx 2,000,000 bacterial genes and 23,000 “Human” genes in you

More microbiome characteristics

- 90% of gut microbiota species belong to 2 phyla: Firmicutes (65%) and Bacteroidetes (25%)
- F:B ratio is ⬆️ in obesity and ⬇️ in IBD
- Likely over 35k species with over 10 Million non-redundant genes
- Influenced by foods among other things

Why our Intestines matter

- Functions as both a barrier and allows specific permeability
  — Both functions affected by bacteria (and by diet)
- Tight junctions and mucous layer provide for permeability or lack thereof
  — Affected by pathogens/bacteria, antibiotics and high fat and/or high sugar diets
  ▪ Potentially aided by pro/pre biotics and fecal transplants

How do WE affect our microbiome

- Genetics
- Vaginal vs C-section delivery
- Breast Feeding versus Formula
- Age
- Environment
- Diet (“Natural” vs processed)
- Medications
- Sleep
- Exercise
- Geography
  — “Healthier” MB
    ▪ Vaginal delivery
    ▪ Breast Feeding
    ▪ Younger age
    ▪ Less processed foods
    ▪ No/Minimal antibiotics
    ▪ No PPIs
    ▪ Higher sleep quality
    ▪ Non-sedentary
Dysbiosis

“Any change to the composition of resident commensal communities relative to the community found in healthy individuals”


Inflammation & Dysbiosis

• Inflammation leads to ▲ in types, diversity and ↑ load of pathogenic bacteria
• Leads to more inflammation…….
• Linked to inflammatory modulated disease  
  — CV, diabetes, arthritis, Alzheimers …
  — Obesity linked to inflammation and then linked to increase risk of a whole host of diseases
  • Weight loss (surgery or diet) helps to normalize microbiome

Importance of Microbial SCFAs

• 3 Principal short-chain fatty acids produced by anaerobic fermentation of dietary fiber by gut bacteria  
  — Acetate
  — Propionate
  — Butyrate

  ▲ Maintain Intestinal Barrier Integrity

• SCFAs are the primary source of energy for intestinal epithelial cells (60-70%)
• ▲ SCFA (esp butyrate) ▲ O2 to intestinal mucosa that favors pathogenic bacteria

Microbiome Alterations Have Been Demonstrated in Common Disorders

• Heart Failure & ASCVD (butyric acid and ↑ trimethylamine-N-oxide)
• Cancer (dysbiosis ▮ immune dysregulation & impaired sensitivity to chemotherapy agents)
• Hypertension (microbial diversity and SCFAs)
• Obesity/Diabetes (diversity/SCFAs, ▲ F:B)
• Depression/Suicidal Ideation (dysregulation of serotonin, dopamine, norepinephrine)

**Diet**

**Negatives**
- Western Diet
- Artificial Sweeteners

**Positives**
- Fiber
- Prebiotics
- Probiotics

**Foods that increase dysbiosis**
- Refined vegetable oils (Omega-6 fatty acids)
- Pasteurized dairy
- Refined carbohydrates
- Conventional meats (due to animal diets)
- Added sugars
- Trans fats

**Foods that maintain a healthy microbiome**
- Fresh fruits and vegetables: full of phytonutrients
  - Whole fruits/veggies: Not juices
- Wild caught fish and cage free eggs, grass raised meat
- Herbs, spices and coffee
- Fermented/active culture foods (sauerkraut, kombucha, miso, yogurt*)
- Probiotics (more later)
- Healthy fats: nuts, seeds, EVOO, coconut oil
- Red wine and dark chocolate in moderation

*Efficacy of yogurt as probiotic depends on how it is processed and may have little to no value

**Quick Pay-off (or quick regression)**
- Microbiome change quickly with diet
- Study published in Nature 2/14 (David et al)
  - Plant vs Animal based diet for 5 days with washout between
  - Gut microbiota changed within 1 day
  - Return to baseline within 2 days
  - Dominant genera seem stable over time, indicating need for stable, healthy diet

**Common Drug Effects**
- Metformin has beneficial affect
  - May be part of beneficial outcomes in diabetes
- Proton Pump inhibitors (e.g. omeprazole)
  - Decrease number and diversity as well as increase GI infections
- **Antibiotics**
  - This is the Big One
  - Rapid/significant changes that last 2-24+ months
  - May kill good and leave bad bacteria

**Ethanol Consumption**
- Linked to intestinal hyperpermeability, endotoxemia and systemic inflammation
- Reduced bacteriodetes and increased pathogenic strains (↑F:B ratio favors insulin resistance & Metabolic Syndrome)
- Small intestine bacterial overgrowth (SIBO) associated with liver disease and diabetes


*Alcohol Res. 2015; 37(2):223–236*
Stress

• Psychosocial stress changes and is changed by neurotransmitters released by the gut microbiome (serotonin, dopamine, GABA)
• Significant variability in individual response to stress and microbiome alterations
• 90% of serotonin is produced in the gut
  – Affects mood and inhibits appetite


Cigarette Smoking

• Cessation profoundly changed microbiome composition (n =10)
• 3-fold increased phylogenetic diversity
• Reduced bacteroidetes and increased firmicutes species


Veteran Diabesity Epidemic

Microbiome/Mycobiome Interaction

EAT !

ADDITION - epiophines (Candida overgrowth)

• Sugar, HFCS
• Fe++, and gluten laden Carbohydrates
• Artificial sweeteners
• Dysbiosis & Candida overgrowth

Bacterial metabolites affect host energy metabolism and appetite....

Other influences

• Sleep
  – Sleep deprivation over 2d period reduces diversity of gut bacteria
• Exercise
  – Animal models show greater number and diversity
  – Human models show the same (may have something to do with other lifestyle elements)
• Gastric Bypass
  – Increase in number and diversity
  – Shift to more beneficial vs harmful bacteria
  – Independent of caloric intake

Fecal Microbiota Transplants (FMT)

• Highly effective for Clostridium difficile (CD) infection – up to 82% cure rate
• Routes of administration: Enema, colonoscopy, nasogastric or nasoduodenal tube or freeze dried capsules
  – Freeze-dried fecal matter in acid-resistant capsules designed to open in the small intestine from screened donors
  – Interestingly: FMT from lean donors seems to help weight loss and from obese donors leads to weight gain
**Human Microbiome Project**

- Set out to examine microbiota in specific locations
- 5 body sites emphasized
  - Oral, skin, gut, lungs, vagina
- Trying to develop a reference set of microbial genome sequences and perform preliminary characterization of the human microbiome
- 242 healthy US volunteers with samples taken from 15 (males) or 18 (females) sites

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**Ocular Microbiome Project**

- Aims to investigate and identify species present on ocular surface (OS) (not including lids)
- Most microbes on OS resist growing in culture
- Demonstration of over 300 bacterial phylotypes on the conj (4x more than thought)
  - 24 bacterial genera never before detected on OS and dozens of unknown species
- About 12 genera dominate conj (1/3 not classified)
- Cornea completely different dozen

**Ocular Microbiome**

- With keratitis, only ½ as many varieties present, prominently Pseudomonas but also overgrowth of staph/strep
  - Changes usually seen BEFORE keratitis diagnosed
- Different microbiome in CL wearers
  - Fewer genera, and Staph dominated

**Gut bacteria & Ocular Defense**

- Gut bacteria presence (or absence) influences ocular microbiome
  - Gut commensal species may serve as a priming signal to generate B-lymphocyte cell repertoires at sites different from the gut
  - Study in mice with control vs those given PO Antibiotic to wipe out gut microbiome
  - IgA in eye-associated lymphatics with AB

- There is a Gut-Eye Axis

**Microbiome and Glaucoma**

- Germ-free mice are spared from ongoing NFL loss after $\uparrow$IOP ($\uparrow$T-cell infiltration/heat shock protein)
- Neurodegeneration is mediated by T cells pre-sensitized to commensal microflora
  - Nat Commun 9; 3009 (2018)
- Oral Microbiome:
  - Missing teeth and alterations in bacteria (higher Strep) were higher in glaucoma cases vs control
  - Pellé et al. Oral microbiome and POAG J Glaucoma 1/17
  - Glaucoma patients have higher bacterial load than controls, possibly leading to neurodegeneration of ONH
  - Astafurov et al. Oral microbiome in OLC. PLoS one. 9/14

**Dry eye**

- OS in normal vs dry eye using rDNA PCR
- Not always a clear cut difference seen
- Both common (Staph) and atypical bacteria found in dry and normal eyes
  - Rhodococcus erythropolis, Klebsiella oxytoca and Erwinia sp were seen in both inflamed and normal eyes
- Increased flora diversity was associated with reduced goblet cell density

Ocular microbiome and dry eye

• Introduction of a probiotic helped to reduce dry eye signs: *Saccharomyces/Enterococcus*
  – TBUT 40%
  – Schirmer's: 45%
  Chiari et al. Dry eye treated w probiotic. Current Clinical Pharm. 7/17.

Gut Microbiome & Eye Disease

SJÖGREN SYNDROME

• Significant MB compositional differences compared to age-matched controls including higher numbers of potentially pathogenic genera
• The severity of SS ocular and systemic disease was inversely correlated with microbial diversity

HLA-B27 associated Uveitis

• Ank Spondylitis, Reiter's, Behcet's, IBS (Crohn's/UC)
• Men > Women
• Link between microbiome and HLA-B27 uveitis
  – Differing theories as to mechanism but believed to be endotoxemia due to loss of gut tolerance
  • Sulfasalazine & methotrexate reduced relapse rates 50% in an observational study of HLA-B27 uveitis
  • MTX improved CME & BCVA
  • Methotrexate reduces commensal bacteria by 40%, especially bacteriodetes species linked to IBD

Microbiome and AMD

• Intestinal microbiome different in AMD vs controls (↑F:B)
  – Both in actual composition and functional elements
  – Corresponding changes in cultured organisms according to diet (as expected)
• Diet affects changes in AMD (in animals)
  – High glycemic vs low glycemic
  – Changes (AMD Features) arrested when switched to LD
• High fat diets that alter gut and increase mAMD independent of weight gain
  – Hypothesized to be due to increased gut permeability and inflammation

Grave’s Disease/Orbitopathy

• Most common systemic autoimmune disease
  – 15% have rheumatoid arthritis (HLA-B27)
• 40% develop Grave’s orbitopathy
• Reduced bacterial diversity with prevalent genera that overlap with RA
  – GD and GO have different prevalent species
  – FMT from GD/GO patients to GF mice ➔ GD/GO
• Serovella, Lactobacillus, Veillonella species
  – F. prausnitzii and Bifidobacteria
• Med Diet may exacerbate (↑Prevotella)
• Treatment alters microbiome/↑diversity
  – Selenium supplements ➔ severity of GO (↓Akermania)

Front. Cell. Infect. Microbiol. 22 December 2021 - thanks to Dr. Linda Morgan!
A Brief, Closer Look at the Microbiome in Diabetes

How Might Gut Bacteria Modulate Diabetes & Diabetic Retinopathy?

• Bacterial translocation (LPS) drives systemic inflammation with macrophage influx into visceral adipose tissue, activation of hepatic Kupffer cells and insulin resistance

• Impaired microbial production of short chain fatty acids impairs intestinal tight junctions while augmenting visceral free fatty acids that worsens insulin sensitivity

Lipopolysaccharides (LPS) are endotoxins derived from the cell wall of gram (-) bacteria

What We Know

• The intestinal flora of patients with diabetes differ from those of patients without diabetes in most analyses (both type 1 and type 2)

• Bacterial diversity is reduced in both type 1 and type 2 diabetes

T1DM/T2DM

• Microbiome diversity is substantially diminished in patients with islet cell auto-antibodies up to a year before the onset of diabetes

• The ratio of Firmicutes:Bacteroidetes is increased 10-FOLD in obesity, Met Synd, T2DM

Stanley Schwartz, MD, FACE
Professor Emeritus of Endocrinology
University of Pennsylvania

Diabetes Egregious 11

Diabetes Care 2015 Jan; 38(1): 159-165
Cell Host Microbe. 2015 Feb 11;17(2):260-73
J Diabetes Metab 2016; 4:253
Nature. 2012 Dec 14;490(7418):55-60
**Leaky Gut Hypothesis in Diabetes**

- Reduction of species that maintain intestinal epithelial tight junctions promotes endotoxemia (translocation of bacteria and lipopolysaccharides) that increases beta cell auto-immunity in T1DM and inflammatory cytokine production linked to T2DM

*Pediatr Diabetes.* 2015 Nov;16(7):485-92

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**Leaky Gut in T2DM Diabetes**

- Endotoxin levels after a high fat meal are 25% higher in obese patients with insulin resistance compared to controls
- Levels are 125% greater in patients with T2DM
- LPS cross though leaky intestinal tight junctions or by infiltrating chylomicrons


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**Reductions of Butyrate Producing Species are Common in T2DM**

- Roseburia species
- Clostridium species
- F. prausnitzii
- A. mucinaphila

*Diabetes Care.* 2015 Jan;38(1):159-65

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**Butyrate in T2DM**

- Introduction of butyrate-producing flora in insulin resistant patients improves insulin sensitivity in the short term

*Gastroenterol October 2015; 143(4): 913–916.e7

- Metformin treatment induces dramatic increases in butyrate-producing Clostridium species and Akermansia muciniphila

*Appl. Environ. Microbiol.* October 2014 vol. 80 no. 19 5935-5943
**A. muciniphila**

- Improves intestinal barrier function, inflammation, insulin sensitivity, fasting blood glucose & triglycerides in humans
  
  *Gut.* 2015 Jan 22; 65(2):398-778

- Pre-biotics with oligofructose increase commensal *A. muciniphila* that prevents diet-induced obesity in mice
  
  Proc Natl Acad Sci U S A. 2013 May 28;110(21):9066-71

  
  *A. muciniphila* increases Treg cells that minimize dymyelination in mouse models of MS
  

**SCFAs and DR?**

- SCFAs, particularly butyrate, inhibit production of inflammatory mediators & proteins linked to diabetic retinopathy
  - NFκB
  - ICAM-1, VCAM-1 and leukocyte adhesion to endothelial cells
  - TNF-a
  - hsCRP

  Arthritis Rheum. 2003 Sep;50(3):697-707
  Inflamm Bowel Dis. 2004 Mar;10(2):122-8

**Fecal Microbiota Transplantation (FMT)**

- 18 obese subjects with T2DM
- Allogenic transplant compared to autologous transplant (n = 9 in each group)
- 79% increased insulin sensitivity at 6 weeks
- 250% increase in Roseburia (butyrate)

**Another Possible Link?**

- Gut-activated T cells cause autoimmune uveitis in mice that is delayed/diminished by antibiotics


- Autoimmune activation of T cells has been implicated in pericyte death underlying DR

  Inflamm Bowel Dis. 2004 Mar;10(2):122-8

  *This raises the possibility that DR could be mitigated by selective destruction of gut-derived pathogenic lymphocytes*

**Symptoms/Signs of Dysbiosis**

- Frequent gas/bloating
- GI cramping/urgency
- Brain fog, anxiety, depression
- Food sensitivities
- Chronic bad breath
- IBS
- Blood glucose abnormalities

**WHAT can we do?**

**Carbohydrate intolerance, esp fiber**
- Hx of prolonged antibiotic use
- Overuse of antacids/PPis
- Autoimmune Dz (MS, Hashimoto’s, psoriasis)
- Sinus congestion

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*Front. Endocrinol., 08 June 2021*
Manipulation of the Microbiome to Reduce the Risk of DM and DR?

- Increase dietary fiber
- Avoid hypercaloric diet and excessive Sat fat
- Avoid artificial sweeteners associated with insulin resistance
- Practice intermittent fasting (improves microbial diversity)
  
  *Wien Klin Wochenschr. 2015 May;127(9-10):394-8*

- Multi-species probiotic supplementation & prebiotics containing oligofructose, chicory root, inulin

Sleep & Exercise

- Sleep deprivation worsens firmicutes:bacteroidetes ratio associated with obesity & insulin resistance in human subjects
  

- Exercise improves firmicutes:bacteroidetes ratio and SCFA production
  
  *Ann Nutr Metab. 2013;63(1-2):1-9*

Value in Probiotics?

- Multi-species probiotic supplementation improved insulin sensitivity & reduced inflammation in humans with T2DM
  — Lactobacillus acidophilus, L. casei, L. rhamnosus, L. bulgaricus, Bifidobacterium breve, B. longum, Streptococcus thermophilus, 100 mg fructo-oligosaccharide
  
  *Ann Nutr Metab. 2013;63(1-2):1-9*

- Meta-analysis shows probiotics reduce fasting glucose, HbA1c, insulin and HOMA-IR in patients with T2DM (11 studies, n=614)
  
  *Br J Nutr. 2016 Apr 14;115(7):1167-77*

Active Culture Probiotic Food Sources

- Yogurt
- Kefir
- Fermented vegetables
- Kimchi
- Pickles
- Sauerkraut
- Miso
- Tempeh
- Kombucha
- Buttermilk
- Fermented cheese [feta, blue, cheddar, goat]

What About Skin Microbes & Diabetes?
Staph Superantigens Cause Diabetes (in rabbits)

- S. aureus exotoxin (toxic shock syndrome toxin-1) causes inflammation, insulin resistance and type 2 diabetes in animals
- Obese patients with T2DM often have hyper-colonization of S. aureus
- Eradication of skin Staph or neutralization of TSST-1 may prevent T2DM

Conclusion

- Rapidly growing field of enquiry across all health care disciplines
  - 87,581 of 109,543 PubMed entries in the last 5 years
- We are what we eat and what our microbiome tells us to eat
- Good health and normal function involve a critical interplay between our behaviors, microbial normalcy, and the gut-brain metabolic axis

Thank You!

Paul Chous
dr_chous@diabeticeyes.com