Bacterial Metabolites: Why We Even Have a Microbiome

Warren Brown, ND
Clinical Science Liaison | Department of Medical Affairs | Genova Diagnostics

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Naturopathic Physician | Clinical Advances for Sport

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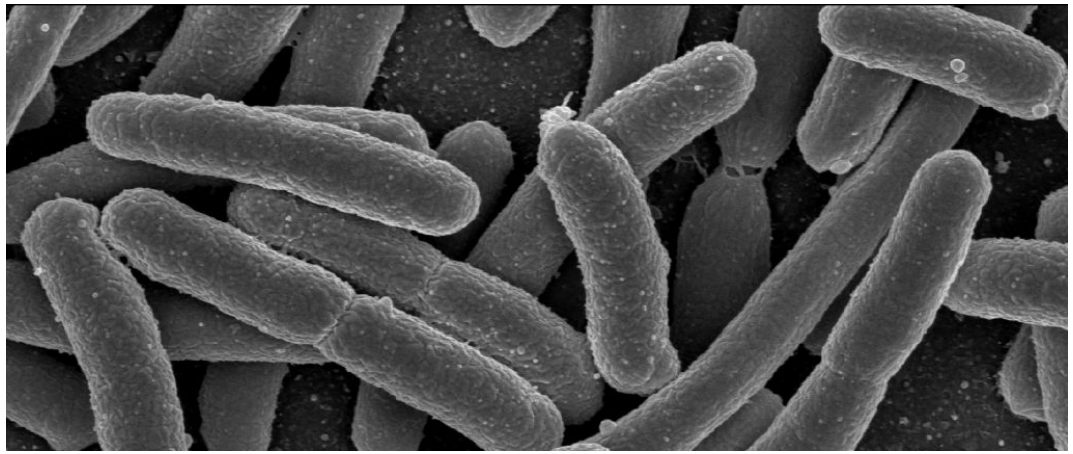
Overview

- Why we have a gut microbiome (symbiosis)
- What are gut microbiome metabolites and why are they important
- Where metabolites fit in the D-I-G framework
- **Interpretation and clinical application**

Why We Have a Gut Microbiome

One of nature's best and most important examples of **symbiosis** (mutually beneficial relationship)

Gut Bacteria Help Us:	We Help Them:
• Digest food • Produce energy • Synthesize nutrients • Modulate the immune system • Protect against pathogens • Produce neurotransmitters	• Stable habitat in GI tract (warm, damp, etc.) • Steady supply of food



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Facts about Metabolites of the Gut Microbiota

- Accounts for approximately 2% of total body mass
- Possess the ability to produce significant amounts of metabolites
  - Hydrolysis
  - Bile acid synthesis
  - **Fermentation**
  - **Enzyme synthesis**
- These metabolites have both local and systemic impacts to human health
  - Vitamins (B-vitamins, vitamin K)
  - Neurotransmitters (serotonin, GABA, dopamine)
  - **Short chain fatty acids (SCFA)**
  - **Enzymes (beta-glucuronidase)**

Some sources suggest that the **metabolic activity of the gut microbiota** rivals that of the **liver**!

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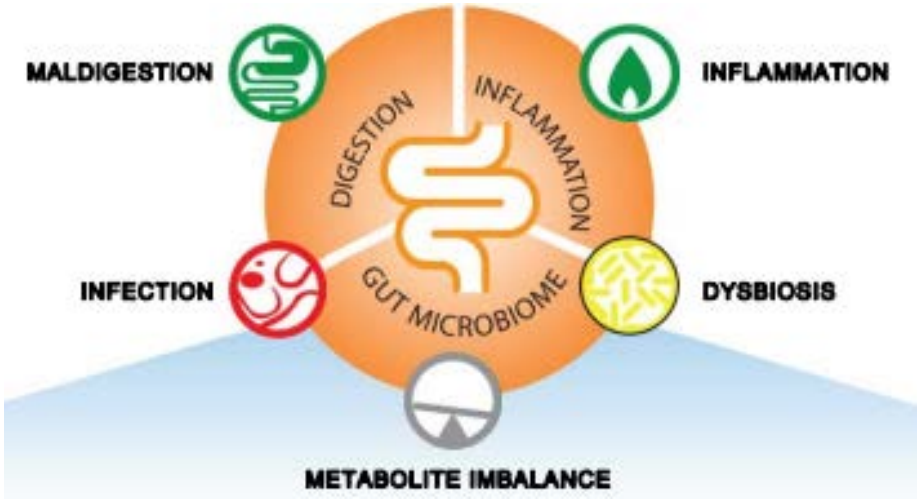
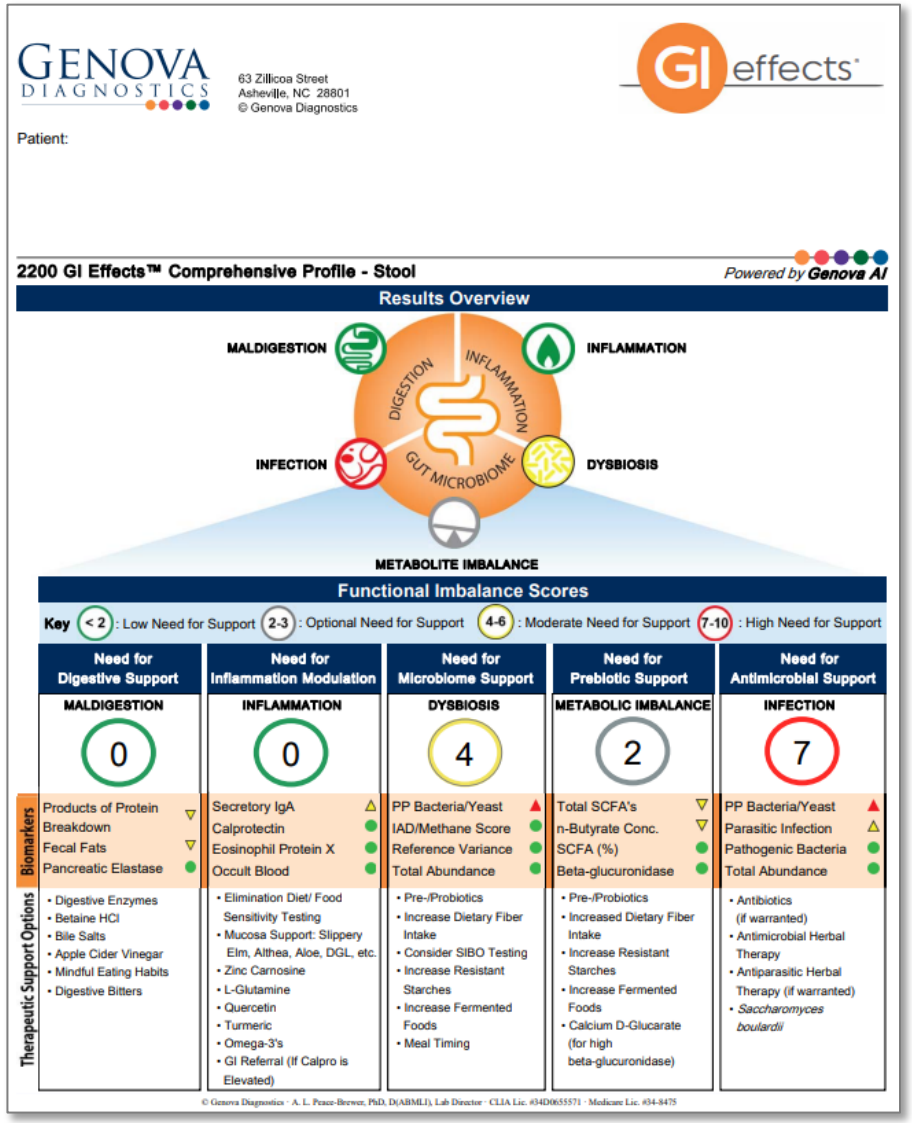
Institute for Functional Medicine, 2019.
Genova's Stool Testing Support Guide, 2023.

Assessment of Gut Function: D-I-G Provides the Framework

D	Digestion/Absorption
I	Inflammation/Immune response
G	Gut microbiome (infection, metabolic imbalance, dysbiosis)

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GI effects

Patient:

2200 GI Effects™ Comprehensive Profile - Stool

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Results Overview

MALDIGESTION
INFLAMMATION
INFECTION
DYSBIOSIS
METABOLITE IMBALANCE
DIGESTION
INFLAMMATION
GUT MICROBIOME

Functional Imbalance Scores

Key: < 2 : Low Need for Support; 2-3 : Optional Need for Support; 4-6 : Moderate Need for Support; 7-10 : High Need for Support

	Need for Digestive Support	Need for Inflammation Modulation	Need for Microbiome Support	Need for Prebiotic Support	Need for Antimicrobial Support
	MALDIGESTION	INFLAMMATION	DYSBIOSIS	METABOLIC IMBALANCE	INFECTION
	0	0	4	2	7
Biomarkers	Products of Protein Breakdown ▽ Fecal Fats ▽ Pancreatic Elastase ●	Secretory IgA △ Calprotectin ● Eosinophil Protein X ● Occult Blood ●	PP Bacteria/Yeast ▲ IAD/Methane Score ● Reference Variance ● Total Abundance ●	Total SCFA's ▽ n-Butyrate Conc. ▽ SCFA (%) ● Beta-glucuronidase ●	PP Bacteria/Yeast ▲ Parasitic Infection △ Pathogenic Bacteria ● Total Abundance ●
Therapeutic Support Options	• Digestive Enzymes • Betaine HCl • Bile Salts • Apple Cider Vinegar • Mindful Eating Habits • Digestive Bitters	• Elimination Diet/ Food Sensitivity Testing • Mucosa Support: Slippery Elm, Althea, Aloe, DGL, etc. • Zinc Carnosine • L-Glutamine • Quercetin • Turmeric • Omega-3's • GI Referral (If Calpro is Elevated)	• Pre-/Probiotics • Increase Dietary Fiber Intake • Consider SIBO Testing • Increase Resistant Starches • Increase Fermented Foods • Meal Timing	• Pre-/Probiotics • Increased Dietary Fiber Intake • Increase Resistant Starches • Increase Fermented Foods • Calcium D-Glucarate (for high beta-glucuronidase)	• Antibiotics (if warranted) • Antimicrobial Herbal Therapy • Antiparasitic Herbal Therapy (if warranted) • *Saccharomyces boulardii*

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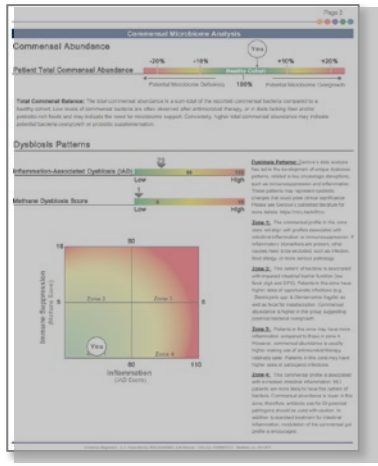
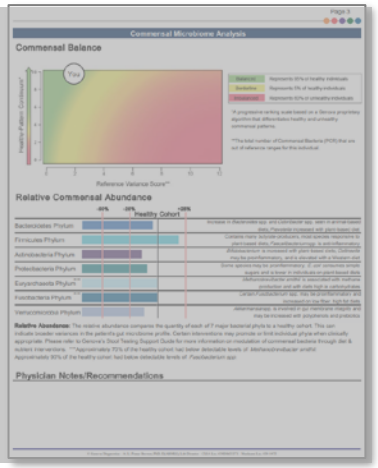
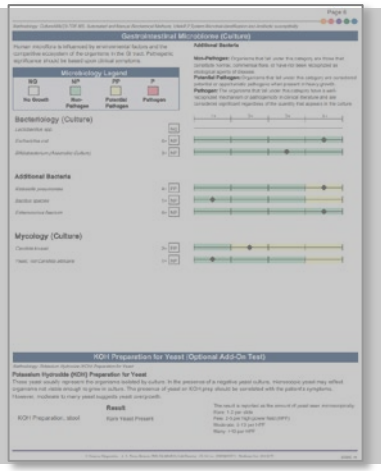
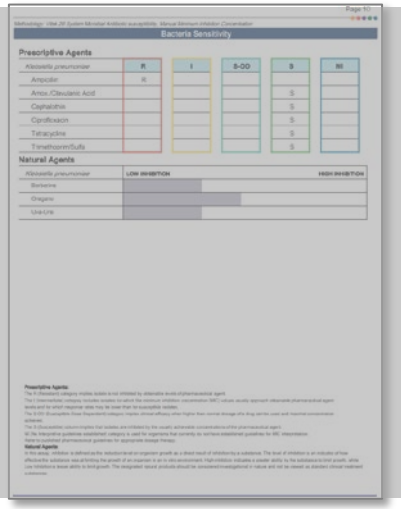
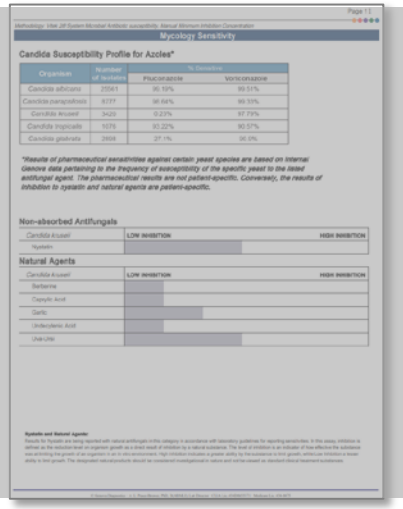
The GI Effects Comprehensive Stool Profile











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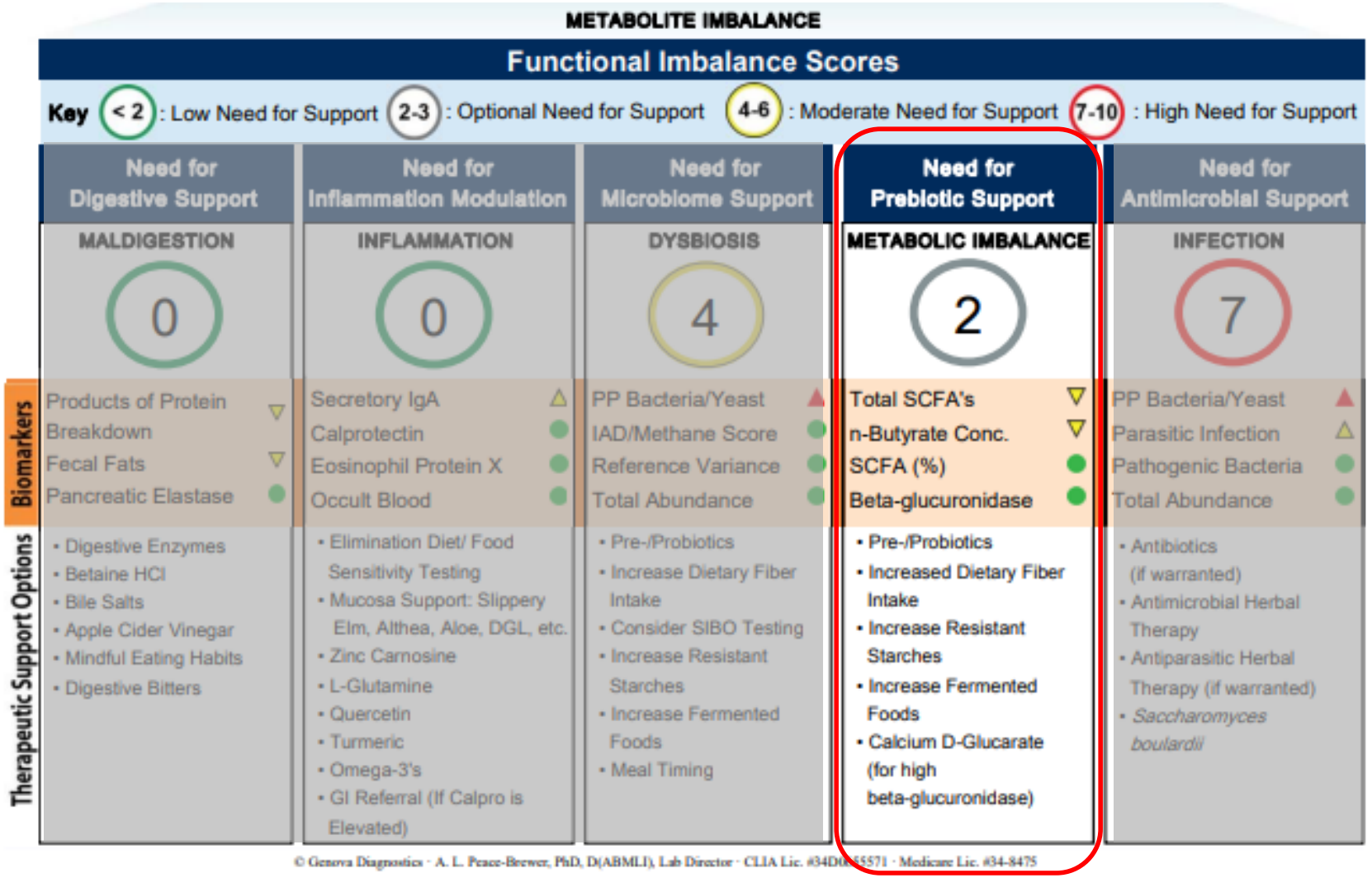
Page 1: Functional Imbalance Scores

- A way of **prioritizing** results with the most significance
- "**Therapeutic Support Options**" are shown on the report



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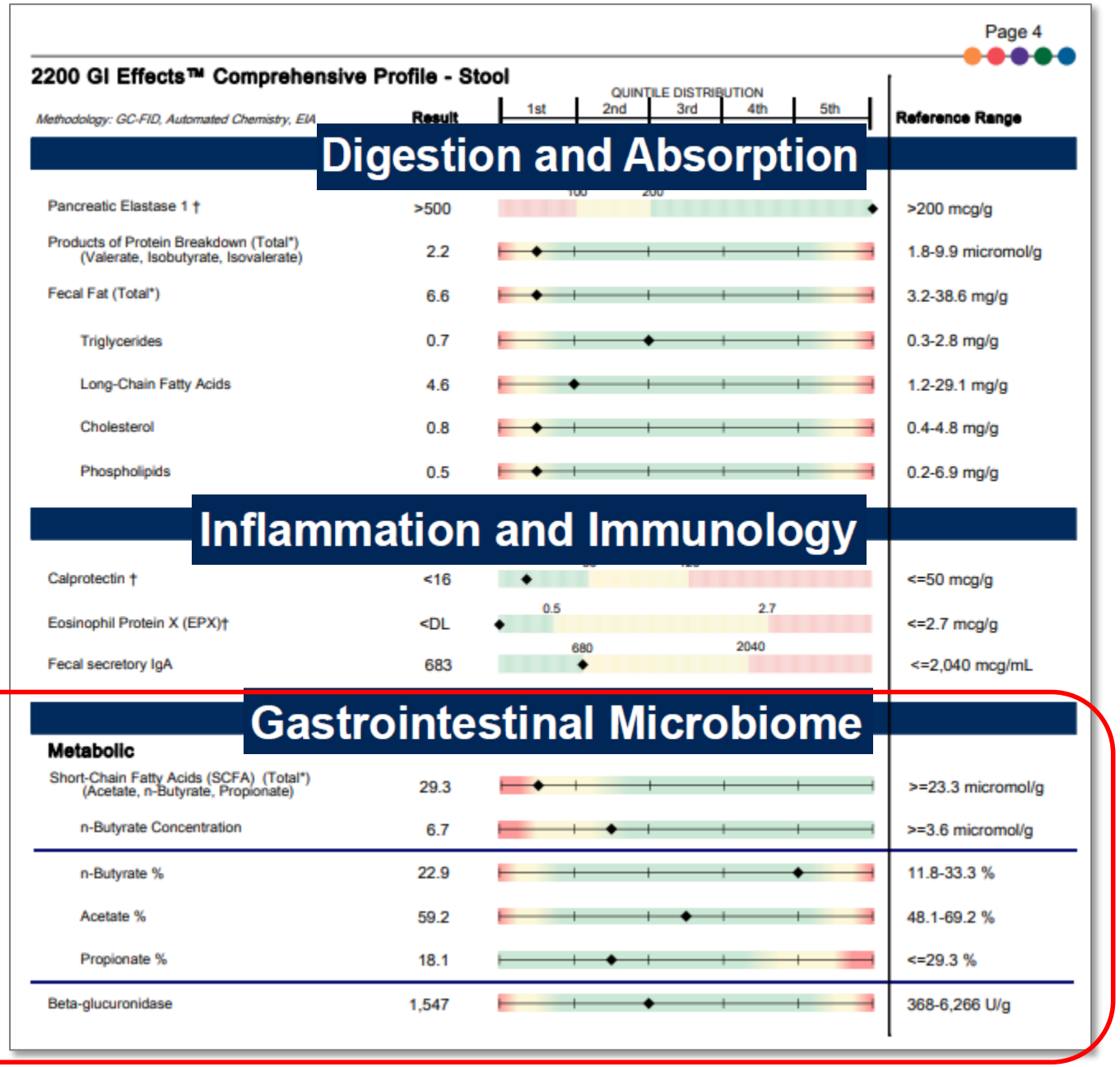
Page 4: Arranged in D-I-G Format

D

I

G



Page 4

2200 GI Effects™ Comprehensive Profile - Stool

Methodology: GC-FID, Automated Chemistry, EIA

	Result	QUINTILE DISTRIBUTION (1st 2nd 3rd 4th 5th)	Reference Range
Digestion and Absorption			
Pancreatic Elastase 1 †	>500		>200 mcg/g
Products of Protein Breakdown (Total*) (Valerate, Isobutyrate, Isovalerate)	2.2		1.8-9.9 micromol/g
Fecal Fat (Total*)	6.6		3.2-38.6 mg/g
Triglycerides	0.7		0.3-2.8 mg/g
Long-Chain Fatty Acids	4.6		1.2-29.1 mg/g
Cholesterol	0.8		0.4-4.8 mg/g
Phospholipids	0.5		0.2-6.9 mg/g
Inflammation and Immunology			
Calprotectin †	<16		<=50 mcg/g
Eosinophil Protein X (EPX)†	<DL	0.5 2.7	<=2.7 mcg/g
Fecal secretory IgA	683	680 2040	<=2,040 mcg/mL
Gastrointestinal Microbiome			
Metabolic			
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	29.3		>=23.3 micromol/g
n-Butyrate Concentration	6.7		>=3.6 micromol/g
n-Butyrate %	22.9		11.8-33.3 %
Acetate %	59.2		48.1-69.2 %
Propionate %	18.1		<=29.3 %
Beta-glucuronidase	1,547		368-6,266 U/g

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Page 4: Gut Microbiome Metabolites

Metabolic			
Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	29.3		>=23.3 micromol/g
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Propionate %	18.1		<=29.3 %
Beta-glucuronidase	1,547		368-6,266 U/g

Need for Prebiotic Support

METABOLIC IMBALANCE

2

- Total SCFA's ▽
- n-Butyrate Conc. ▽
- SCFA (%) ●
- Beta-glucuronidase ●

- Pre-/Probiotics
- Increased Dietary Fiber Intake
- Increase Resistant Starches
- Increase Fermented Foods
- Calcium D-Glucarate (for high beta-glucuronidase)

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Microbiome Metabolite Interpretation

- Best interpretation is achieved by keeping the clinical context in mind
- The following aspects of a patient's clinical history are helpful to know:
  - **Diet**: fiber intake, fermented foods
  - **Supplements**: probiotics, prebiotics, butyric acid
  - **Medications**: antibiotics, laxatives
  - **Transit time**: fast (diarrhea) or slow (constipation)
  - **Symptoms**: gas/bloat, SIBO symptoms

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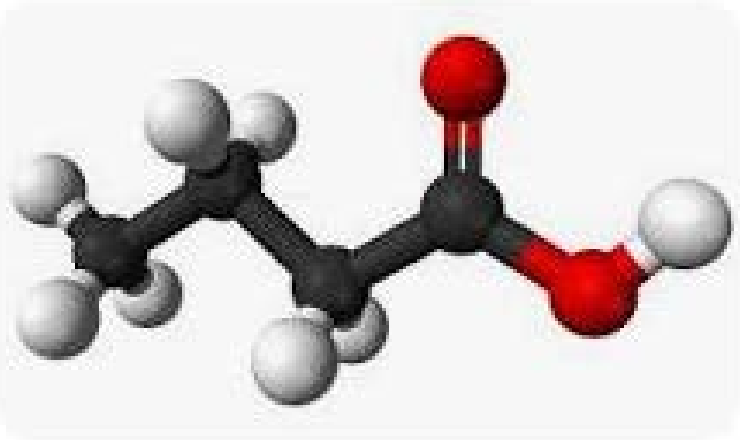
Photo credits: Creative Commons.

Short Chain Fatty Acids (SCFA)

- SCFAs are organic acids containing one to six carbons
- Acetate, propionate, and butyrate are the most abundant (≥95%)
- They are **produced** by **bacterial fermentation** of **prebiotics** (dietary fiber and/or resistant starch)
- There are also some anaerobic bacteria that can produce SCFAs from endogenous epithelial-derived mucus, but this is thought to be a minor contributor



Butyric Acid
($C_4H_8O_2$)

Resistant starch – a type of carbohydrate that is 'resistant' to digestion in the small intestine, but is eventually **fermented by commensal bacteria** in the **large intestine**

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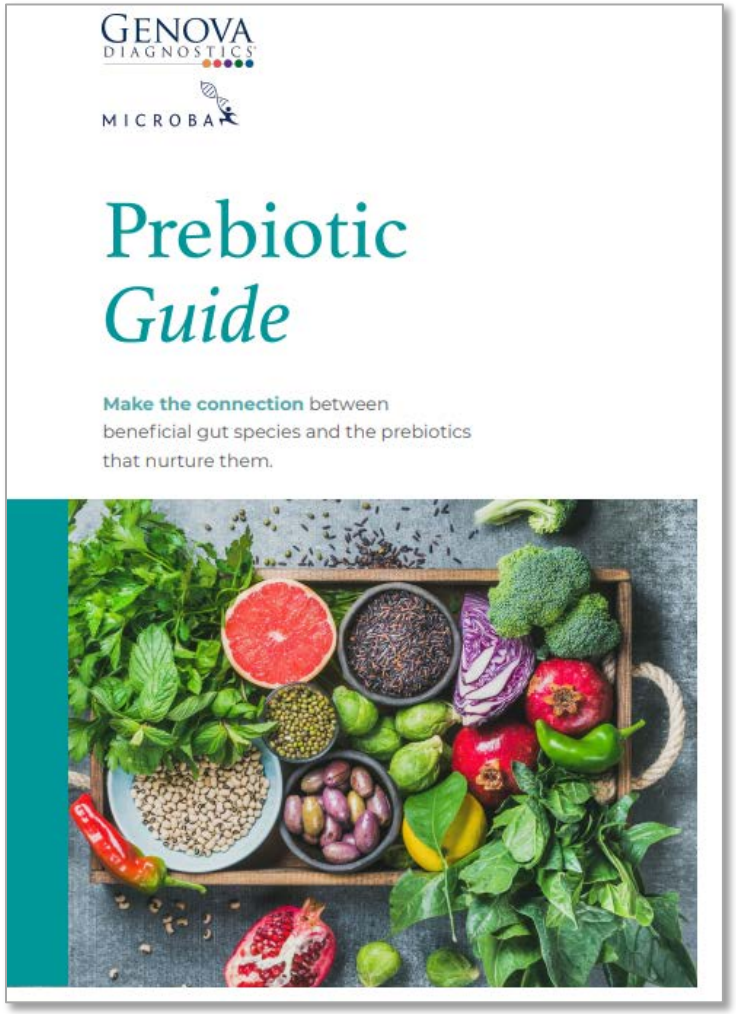
Photo credit: Wikipedia.
Genova's Stool Testing Support Guide, 2023.

Genova's Prebiotic Guide

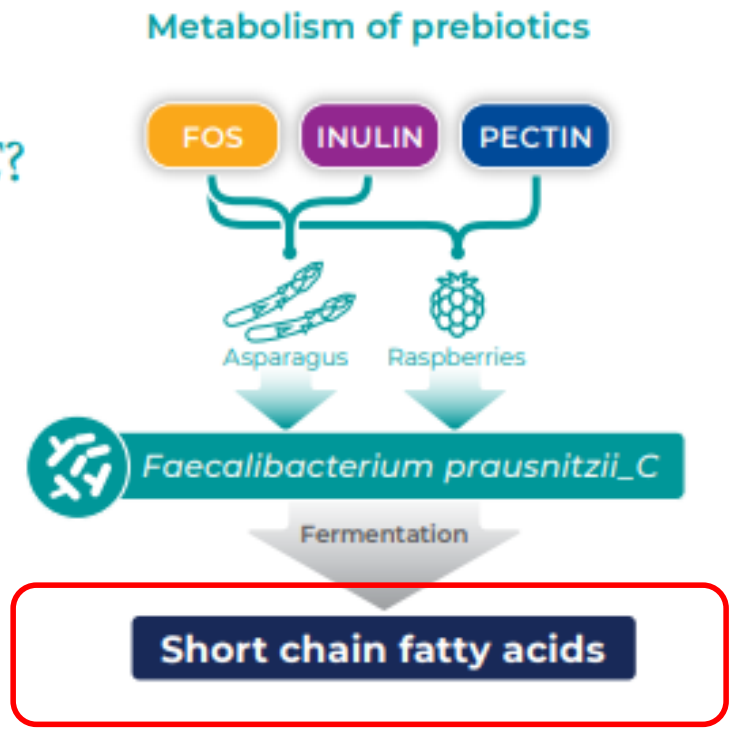


What's on the menu for *Faecalibacterium prausnitzii_C*?

FOS (Fructooligosaccharides): Pistachios, Pumpernickel Bread, Red Lentils

INULIN: Barley, Whole Wheat Pasta, Ripe Bananas

PECTIN: Butternut Pumpkin, Green Peas, Sweet Potatoes

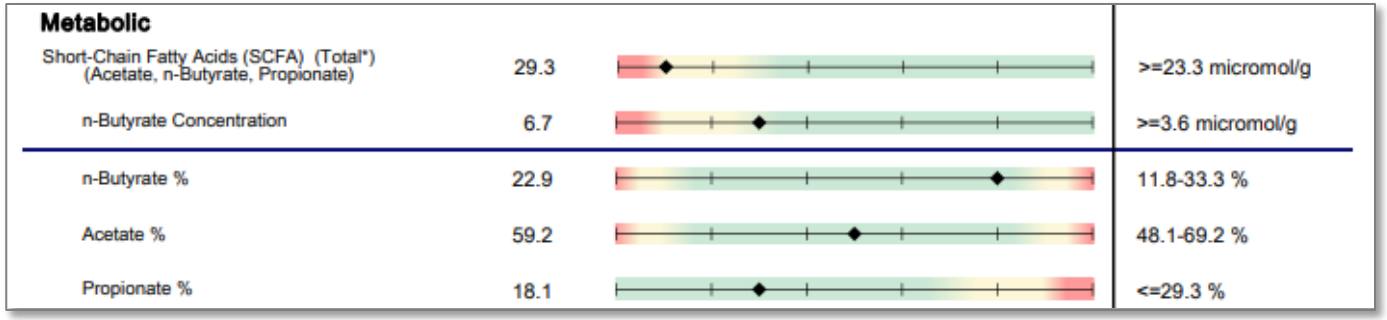


https://www.gdx.net/core/supplemental-education-materials/gdx--microbiomix-prebiotic-guide.pdf

SCFA as “Post-biotics”

SCFA functions

1. Maintain **intestinal barrier** function
2. Provide **fuel** for colonocytes
3. Regulate colonic **absorption** of water, electrolytes, and nutrients
4. Support commensal **bacteria**
5. Modulate **anti-inflammatory** and **antimicrobial** activities, as well as some aspects of **immunity**



Post-biotics

- **Substances produced by commensal organisms** that provide health **benefits to the host**
- **Metabolic byproducts** or bacterial components such as vitamins, amino acids, antimicrobial peptides, and **SCFA**s

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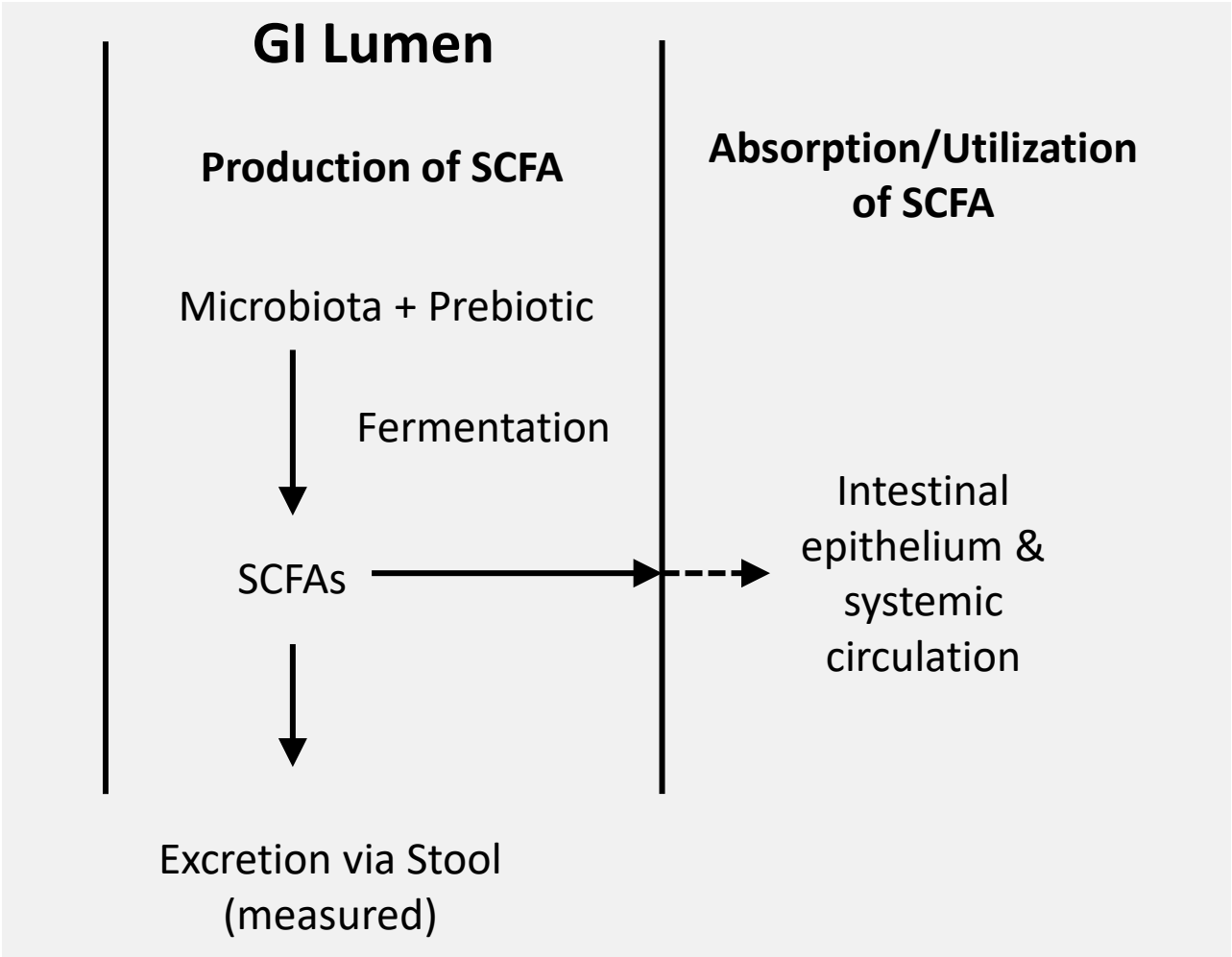
Genova’s Stool Testing Support Guide, 2023.
Venegas, D. et al. Front. Immunol., Short Chain Fatty Acids (SCFAs)…11 March 2019.

Interpretation: Total SCFA

- Total SCFAs LOW:
  - Low production *and/or*
  - High absorption/utilization
- Total SCFAs HIGH:
  - High production *and/or*
  - Low absorption/utilization

- ***Increased*** **absorption/utilization** of SCFA may occur in **response to inflammation** during some stages of the healing process in the intestinal wall



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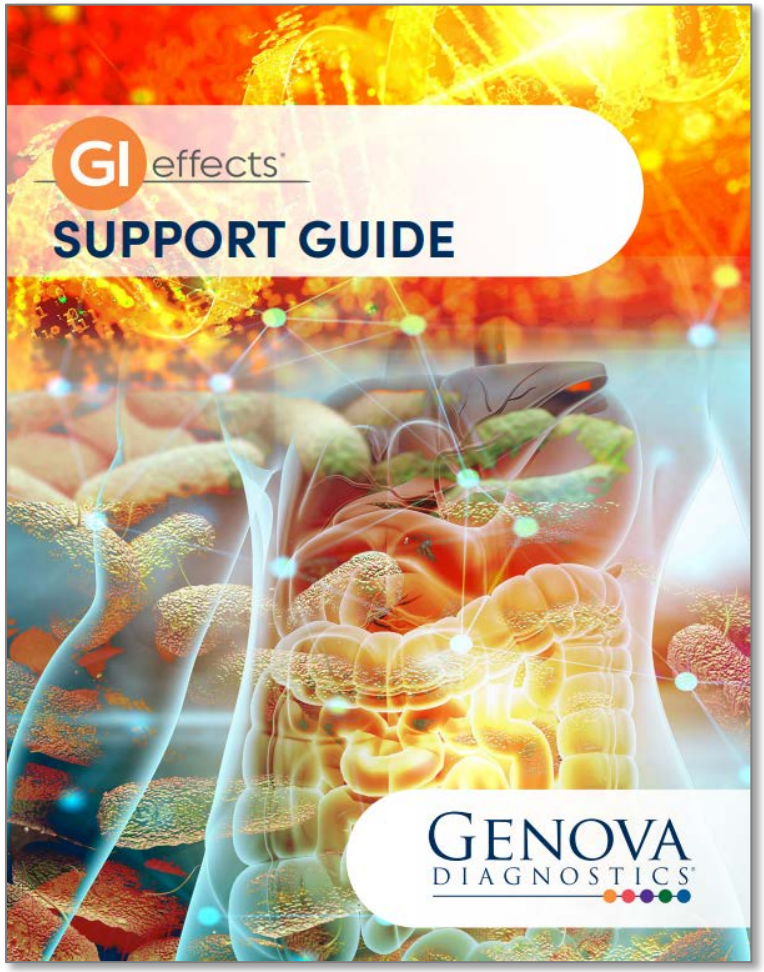
Venegas, D. et al. Front. Immunol., Short Chain Fatty Acids (SCFAs)…11 March 2019.

Interpretation: n-Butyrate concentration

- Butyrate is the primary fuel source for colonocytes
- Inadequate levels are associated with disordered colonic health
- Published literature mentions *Faecalibacterium*, *Eubacterium*, and *Roseburia* as major butyrate producers
- Various mixtures of dietary fibers, some types of resistant starch, fructooligosaccharides (FOS), and beta-glucan are important substrates for butyrate production

- While the **n-butyrate concentration** is the measured level, the **n-butyrate %** shows its relationship to the total SCFA – they are not always going to be in the same position on their reference ranges



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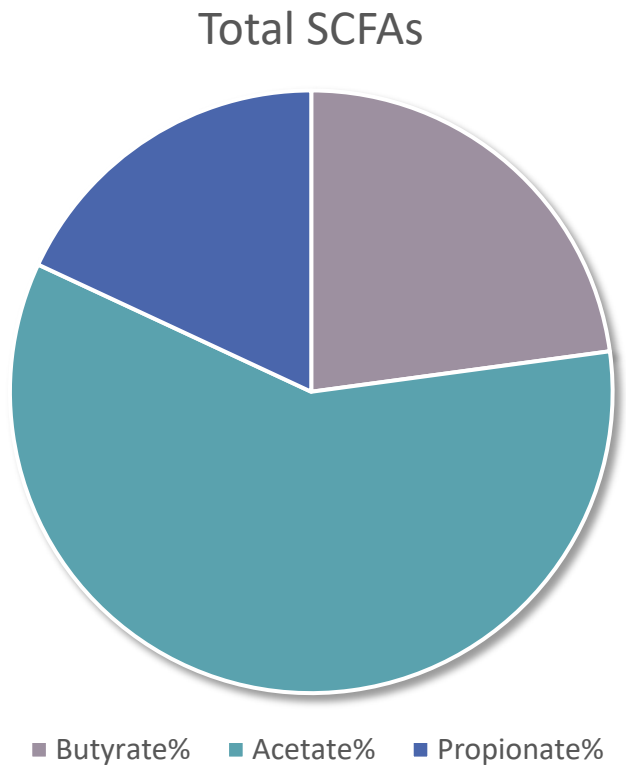
Interpretation: Percentages of SCFAs

- **Imbalanced percentages** of the individual SCFAs may reflect an **imbalanced microbiome** or **diet**
- SCFA percentages are **indirect indicators** of altered intestinal microbial composition



n-Butyrate %	22.9		11.8-33.3 %
Acetate %	59.2		48.1-69.2 %
Propionate %	18.1		<=29.3 %

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Why Might the % of SCFA be Imbalanced?

Literature-Based Short Chain Fatty Acid Production		
Butyrate Producer (C4:0)	**Acetate Producer (C2:0)**	**Propionate Producer (C3:0)**
F. prausnitzii *B. crossotus* *A. colihominis* *Clostridium* spp. *C. eutactus* *Roseburia* spp. *B. uniformis*	*Prevotella* spp. *Odoribacter* spp. *A. colihominis* *Clostridium* spp. *C. eutactus* *Lactobacillus* spp. *R. bromii* *Veillonella* spp. *Bifidobacterium* spp. *A. muciniphila*	*Phocaeicola vulgatus* *Prevotella* spp. *Odoribacter* spp. *Clostridium* spp. *Veillonella* spp. *A. muciniphila*
Butyrate Utilizer	**Acetate Utilizer**	**Propionate Utilizer**
Clostridium spp.	*Roseburia* spp.	*Clostridium* spp.

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SCFA Interpretation: Example 1

Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	25.7		>=23.3 micromol/g
n-Butyrate Concentration	7.7		>=3.6 micromol/g
n-Butyrate %	30.0		11.8-33.3 %
Acetate %	48.6		48.1-69.2 %
Propionate %	21.5		<=29.3 %

- Borderline low total SCFA (not many SCFA present in the stool)
- Most SCFA found consisted of n-butyrate, but don't forget about the total SCFA
- May be the result of **suboptimal intake of dietary fiber / resistant starch**, or **suboptimal commensal bacteria**
- May be best to address the areas above; also consider increasing intake of inulin or pectins (substrates for acetate-producing bacteria)

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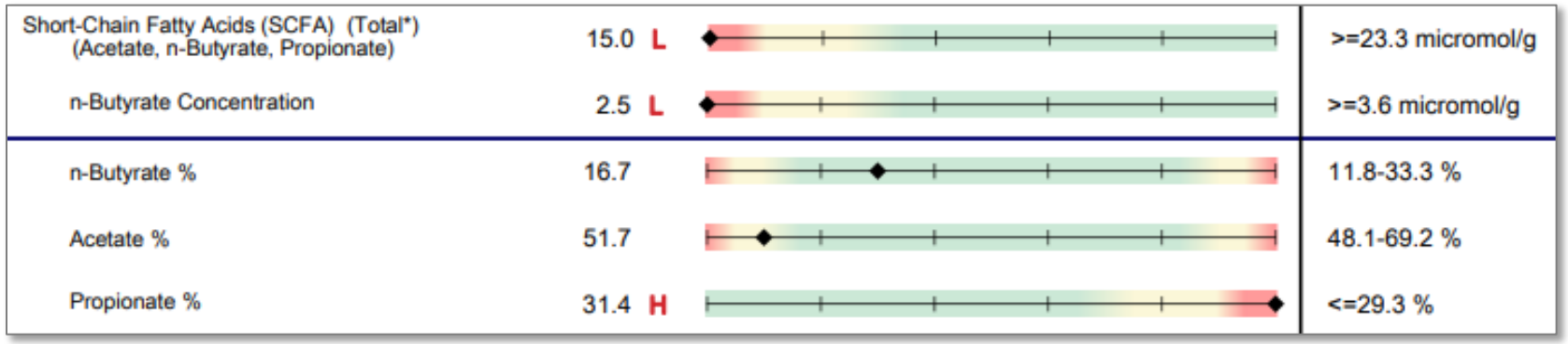
SCFA Interpretation: Example 2



Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	15.0 L		>=23.3 micromol/g
n-Butyrate Concentration	2.5 L		>=3.6 micromol/g
n-Butyrate %	16.7		11.8-33.3 %
Acetate %	51.7		48.1-69.2 %
Propionate %	31.4 H		<=29.3 %

Causes of low SCFAs

- Diarrhea (rapid transit leading to decreased SCFA production)
- Constipation (increased SCFA absorption)
- Inflammation (high calprotectin and/or high EPX/sIgA)
- Chronic antibiotic use
- Decreased carbohydrate/fiber consumption[130-132]
- Chronic illness with restricted diet (e.g., low fermentable fiber)
- Severe dysbiosis (e.g., some commensal bacteria are very high, while others are very low)

Therapeutic considerations for low SCFAs

- Dietary fiber, resistant starches (e.g., seeds and legumes, whole grains, green bananas, potatoes) and/or butyrate supplementation
- Arabinogalactans and β-glucan, as found in whole-grains[133]
- Inulin supplementation[128]
- Probiotics and fermented foods to balance the microbiome

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SCFA Interpretation: Example 3

Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	45.6		>=23.3 micromol/g
n-Butyrate Concentration	6.0		>=3.6 micromol/g
n-Butyrate %	13.2		11.8-33.3 %
Acetate %	77.7 H		48.1-69.2 %
Propionate %	9.2		<=29.3 %

Acetate

Acetate is the most abundant SCFA in the colon and makes up more than half of the total SCFAs.

Acetate has two main routes of production. The primary route is carbohydrate fermentation by enteric bacteria. Acetate is formed directly from acetyl-CoA, gets released into systemic circulation, and is taken up by the liver. It is then used as an energy source, as well as a substrate for the synthesis of cholesterol and long-chain fatty acids.[126]

Acetate is recognized as a volatile signal for biofilm formation.[127]

Inulin supplementation has been shown to increase acetate levels.[128] Pectin is also an important substrate for acetate production.[125]

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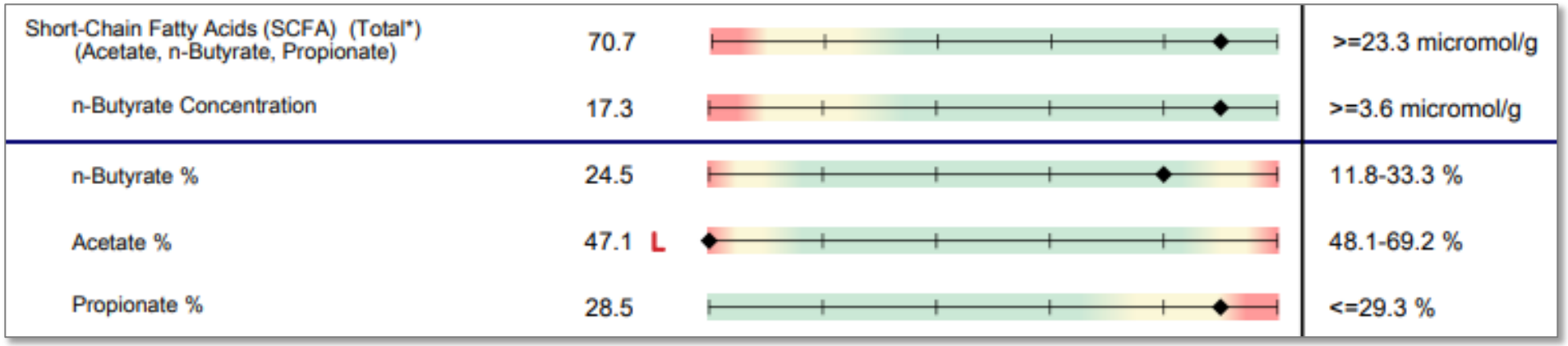
SCFA Interpretation: Example 4



Short-Chain Fatty Acids (SCFA) (Total*) (Acetate, n-Butyrate, Propionate)	70.7		>=23.3 micromol/g
n-Butyrate Concentration	17.3		>=3.6 micromol/g
n-Butyrate %	24.5		11.8-33.3 %
Acetate %	47.1 L		48.1-69.2 %
Propionate %	28.5		<=29.3 %

Causes of elevated SCFAs

- Elevated commensal bacteria abundance or bacterial overgrowth[134]
- High dietary intake of fiber and resistant starches

Optimal levels of SCFAs have not been established. However, in general, higher levels are considered beneficial.

Therapeutic considerations for high SCFAs

- May be optimal
- Consider SIBO testing if any of these apply
  - Total abundance of commensal bacteria is high
  - Products of Protein Breakdown are elevated
  - Fecal fats are elevated
  - *Methanobrevibacter smithii* is high via qPCR

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Beta-glucuronidase

Beta-glucuronidase	1,547		368-6,266 U/g

Beta-glucuronidase is an enzyme which is produced by colonocytes and by some intestinal bacteria (particularly *E. coli*, but also *Ruminococcus*, *Bacteroides*, *Eubacterium*, *Peptostreptococcus*, *Staphylococcus*, and *Clostridium*).[135]

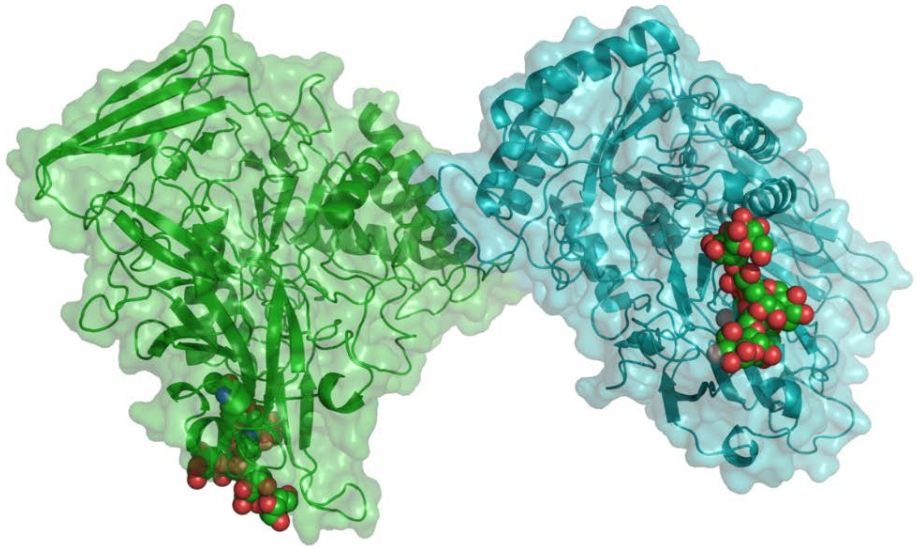


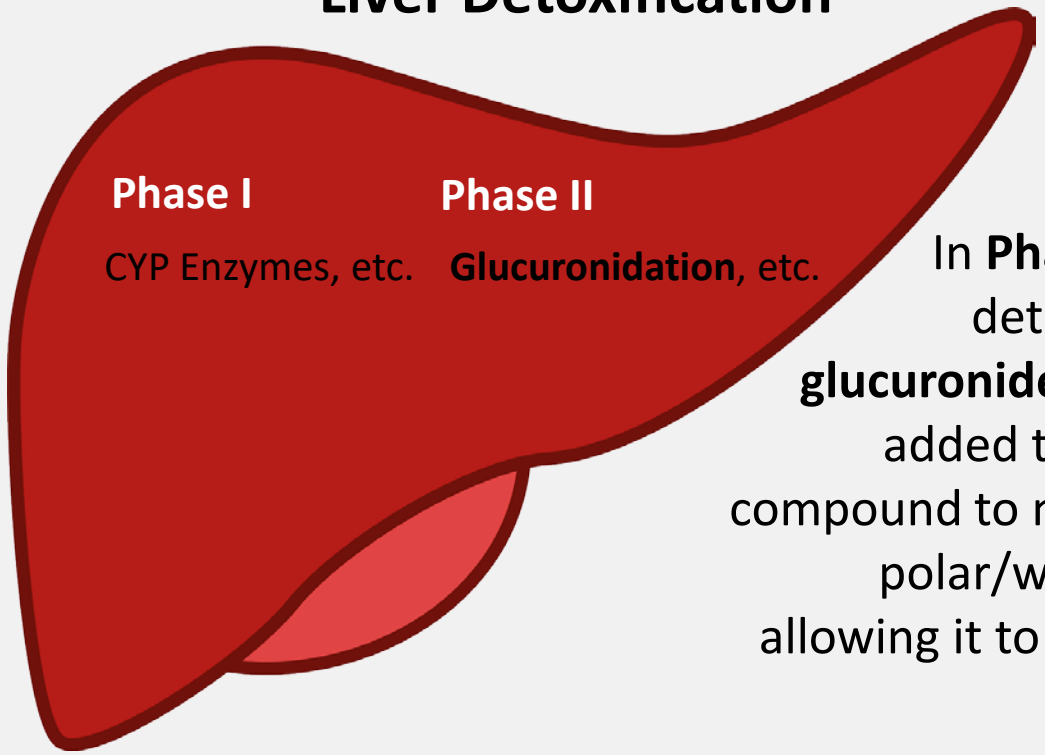
Image from the German Wikipedia page for Beta-glucuronidase.
Genova's Stool Testing Support Guide, 2023.

Beta-glucuronidase

Beta-glucuronidase	1,547		368-6,266 U/g

Helpful Tip: The two most important parts of the word:

- "***glucuronid***" – relating to glucuronidation
- "***ase***" – it's an enzyme



In **Phase II** of liver detoxification, a **glucuronide** molecule is added to a chemical compound to make it more polar/water soluble, allowing it to be excreted.

Beta-glucuronidase

Beta-glucuronidase	1,547		368-6,266 U/g

- **Reverses** the **conjugation step** in **Phase II of liver detoxification** by uncoupling glucuronides
  - Promotes enterohepatic **recirculation** of **toxins**, **hormones**, and **some medications**
- A **moderate level of beta-glucuronidase activity is preferred** as activity appears to be important for **normal enterohepatic** recirculation of:
  - Endogenous compounds
  - Phytonutrients/polyphenols
  - Vitamins (mixed literature support)
- Human studies associate **high** beta-glucuronidase to colon cancer and hormone-related cancers

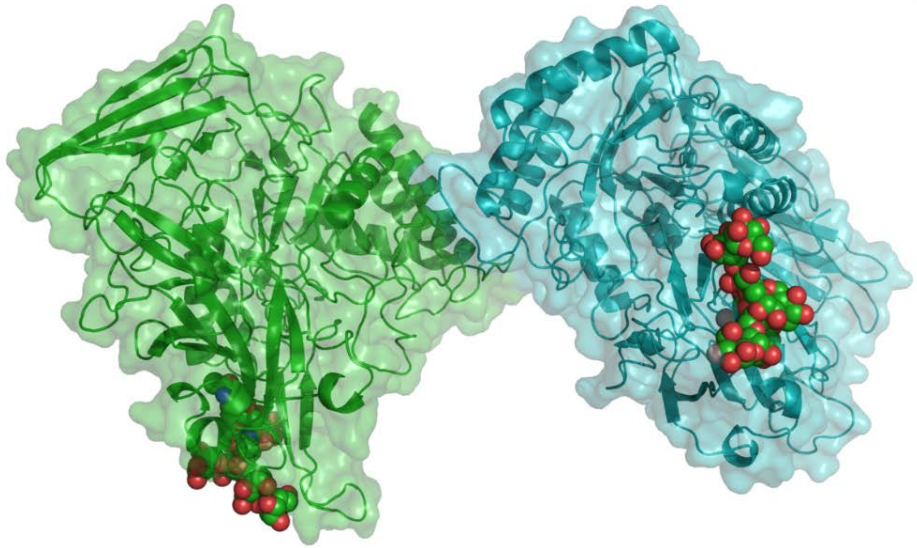


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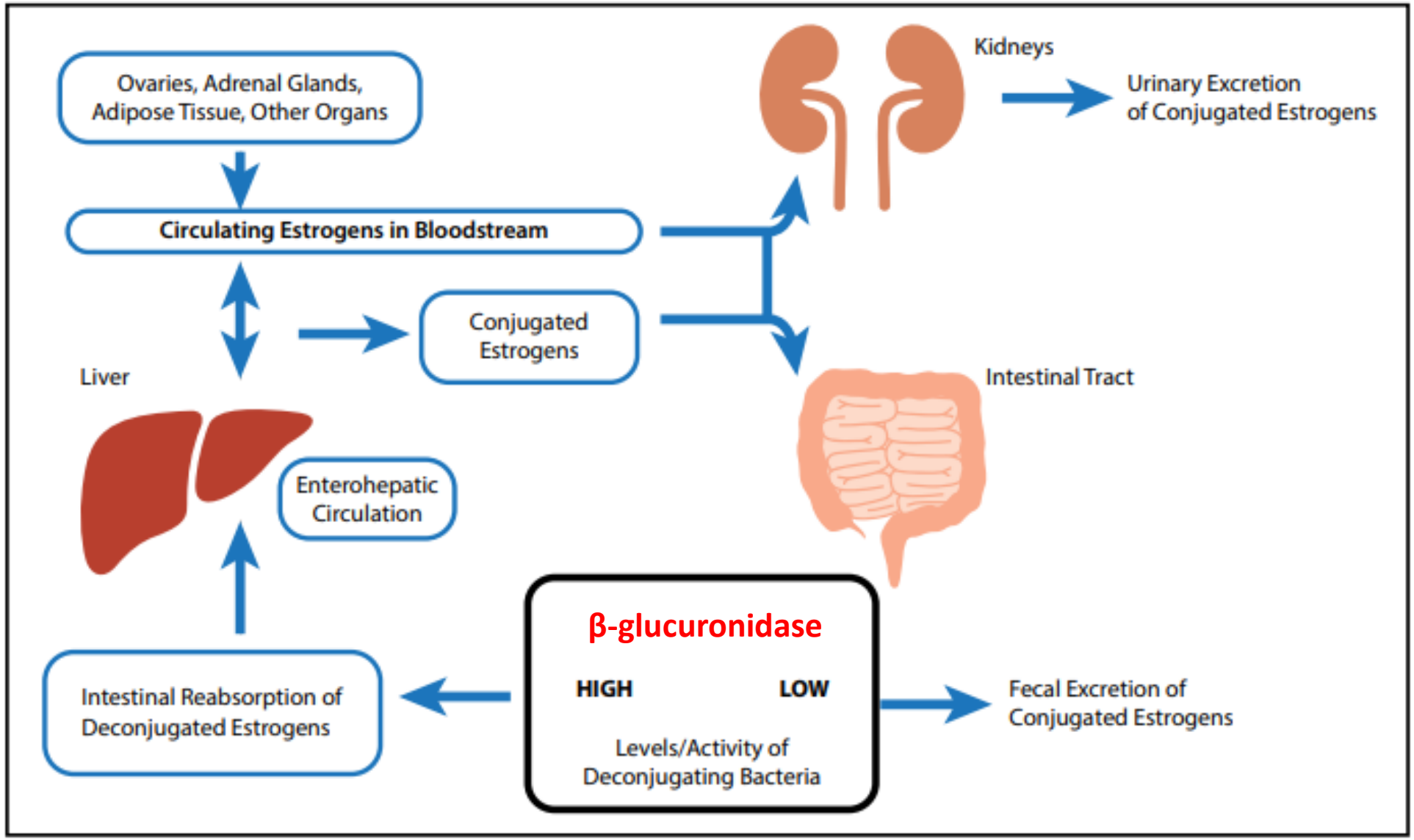
Image from the German Wikipedia page for Beta-glucuronidase.
Genova's Stool Testing Support Guide, 2023.

Beta-glucuronidase: Estrogen Example



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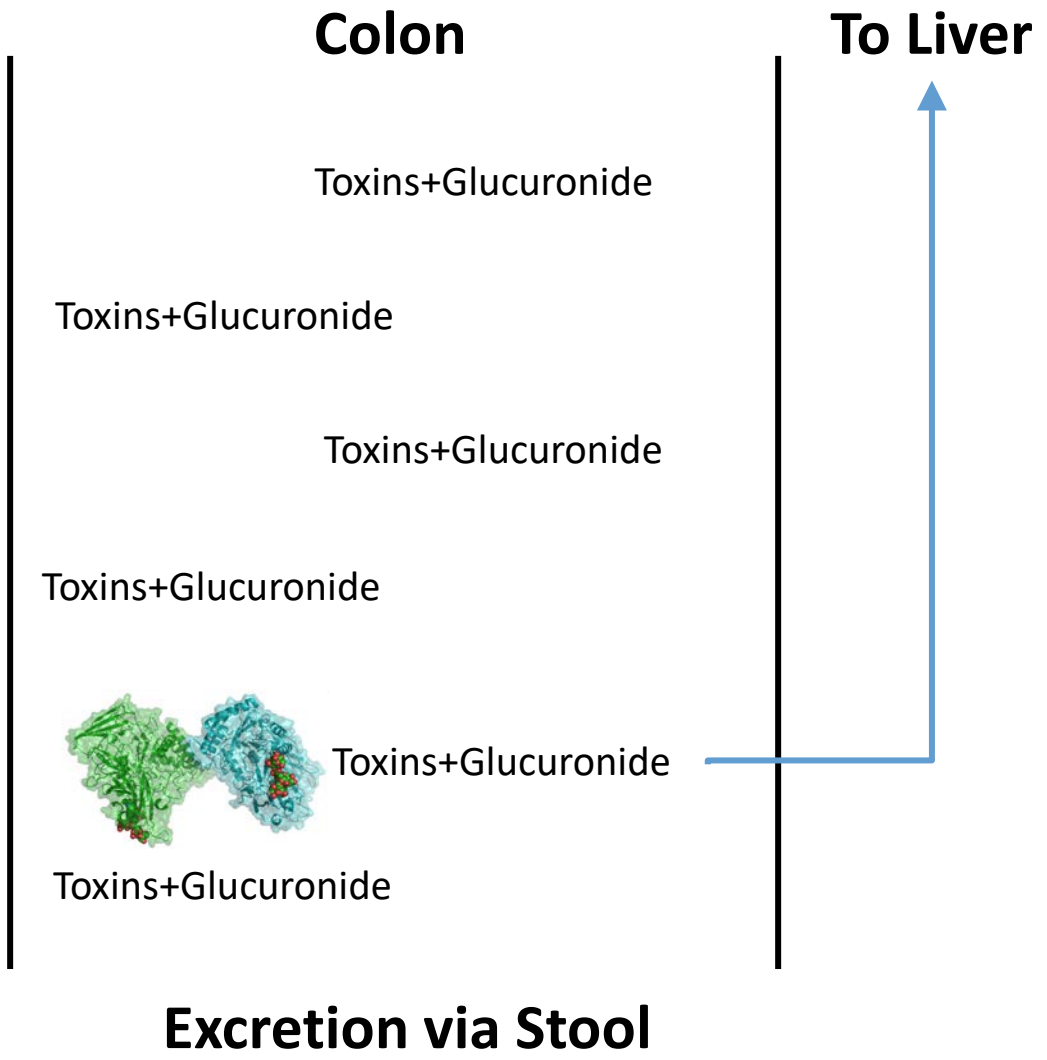
Beta-glucuronidase Interpretation: LOW Levels

Causes of low beta-glucuronidase

- Dysbiosis
- Antibiotic use[151,152]

Therapeutic considerations for low beta-glucuronidase

Abnormally low levels may diminish the bioavailability of many phytonutrients. There is no literature indicating the need to treat low fecal β-glucuronidase. However, because it is produced in the intestinal endothelium and by commensal bacteria, maintaining a healthy commensal balance may be helpful to optimize levels.



GENOVA DIAGNOSTICS

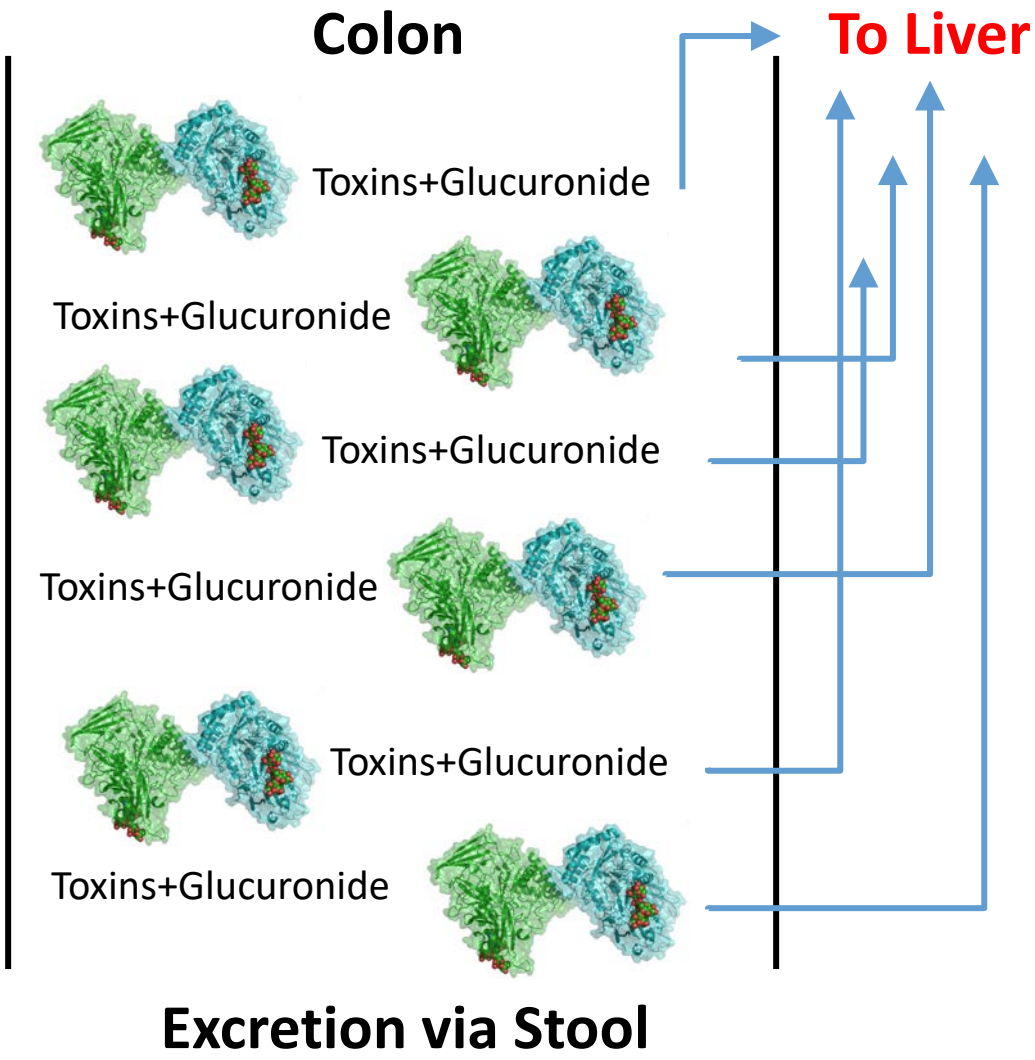
Beta-glucuronidase Interpretation: HIGH Levels

Causes of elevated beta-glucuronidase

- Dysbiosis
- Western diet, high in red meat and protein[135,142]

Therapeutic considerations for elevated beta-glucuronidase

- Probiotics[143,144]
- Dietary fiber, prebiotics[143-146]
- Calcium-D-glucarate
  - » Calcium-D-glucarate is the calcium salt of D-glucaric acid. It is found in fruits and vegetables (oranges, apples, grapefruit, and cruciferous vegetables).[147]
  - » Oral supplementation inhibits the enzymatic activity of beta-glucuronidase [147]
- Milk thistle[148,149]
- Low-calorie and vegetarian diets[135,150]



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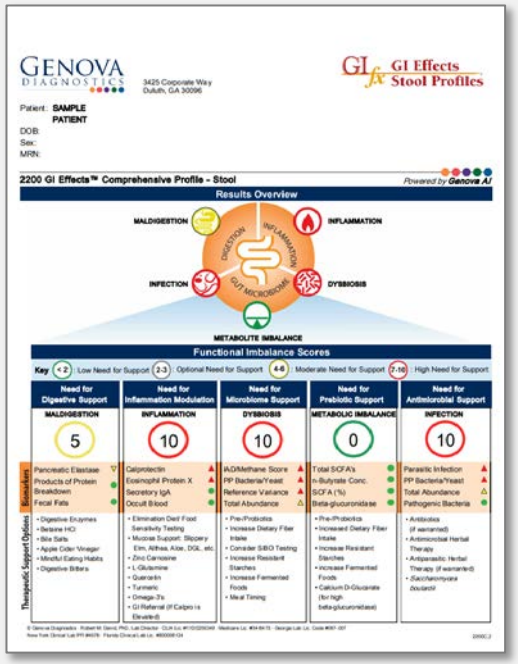
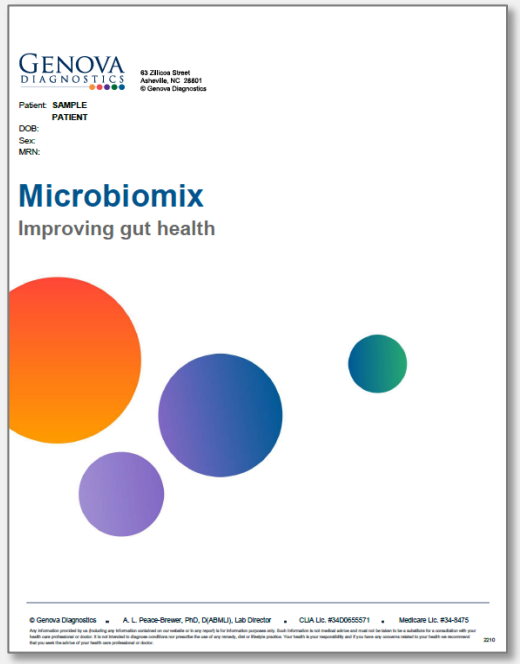
To Dive Deeper into Assessment of Microbial Metabolites, Consider Genova's *Microbiomix* add-on



- Multiple methodologies, including qPCR
- 6 metabolites measured directly



- Whole genome sequencing, allowing for ID of >95% of the entire microbiome
- Genetic potential of microbiome's potential to produce 13+ metabolites

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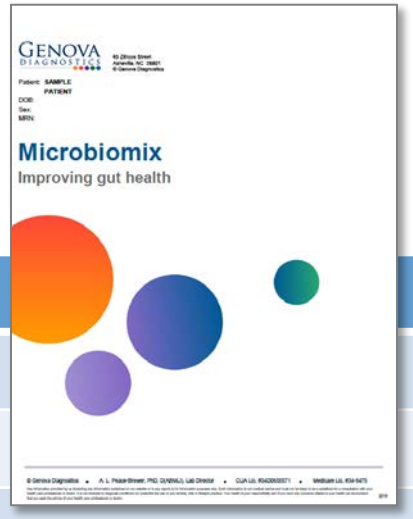
Metabolites Assessed in Genova's *Microbiomix*



Metabolite	Clinical Considerations
Hexa-LPS	Contributor to inflammation
Trimethylamine	Cardiovascular risk factor (TMAO)
Methane & Hydrogen Sulfide Gas	Consideration for SIBO testing
Ammonia (Urease)	Protein recycling and risk for IP; consider Lactulose/Mannitol testing
B. fragilis toxin	Potential for infectious diarrhea
Beta-glucuronidase	Potential for excessive recirculation of toxins & steroid hormones
Oxalate consumption	Association with calcium oxalate kidney stones; consider additional testing
Neurotransmitters (GABA, IPA, Histamine)	Additional insight into gut-brain axis
SCFAs	Important for health of colonocytes
Vitamin production	Potential for GI synthesis of nutrients; consider NutrEval/Metabolomix

Genova Quality

With regards to the **validity** and **reproducibility** of our results, we are licensed by CLIA, the federal agency regulating laboratories, as well as by those states requiring individual licenses.

Additionally, we participate in several **external proficiency testing programs**, such as CAP, WSLH, and QMEQAS which help to maintain the accuracy of our laboratory assays. **Internally** we conduct on-going assessments, including inter-assay precision, analytical sensitivity, interference studies, etc. to ensure we continue to meet laboratory quality standards.

All results released are covered by **extensive quality controls**.

Proficiency Participation

Asheville/Atlanta

- CAP (College of American Pathologists)
- NY State
- WSLH (Wisconsin State Laboratory of Hygiene)
- PA State
- QMEQAS (Quebec Multielement External Quality Assessment Scheme)
- IBL (Innovation Beyond Limits) International

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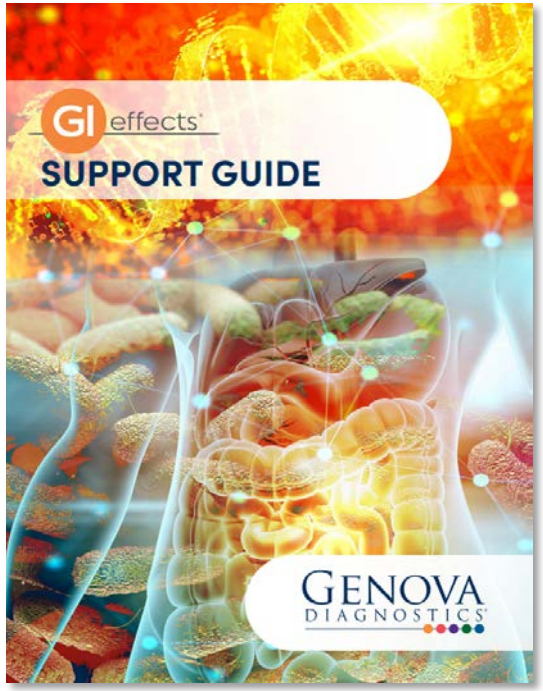
Additional Educational Resources



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Thank you for your time and attention!