Precision Probiotics

For Healthcare Providers
Utilizing Targeted Strains

Christina O'Connor, RD
Healthcare Sales Account Manager, Pendulum

Megan Landrum, MS, RD

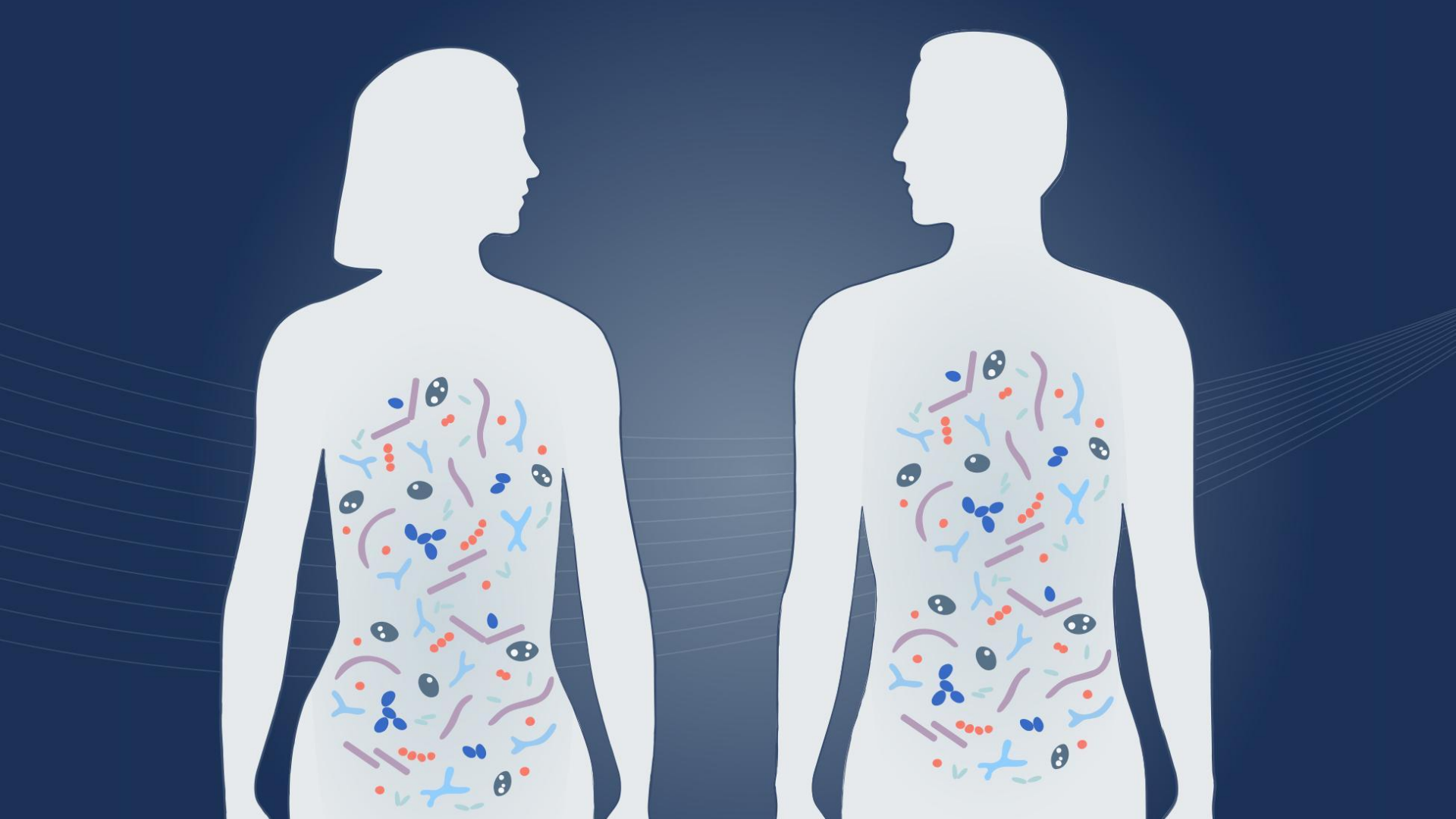
Megan Landrum is a Registered Dietitian and VP of Healthcare Programs at Pendulum. She received her bachelor of science in Nutrition and Dietetics from Arizona State University and completed her Dietetic Internship at Oregon Health and Science University. Megan went on to complete a Master's in Human Nutrition and Functional Medicine from the University of Western States.

Megan has worked in both clinical and private practice settings, but in the past 10 years has found a passion for the gut microbiome, leading healthcare education and implementation of microbiome-targeted solutions.

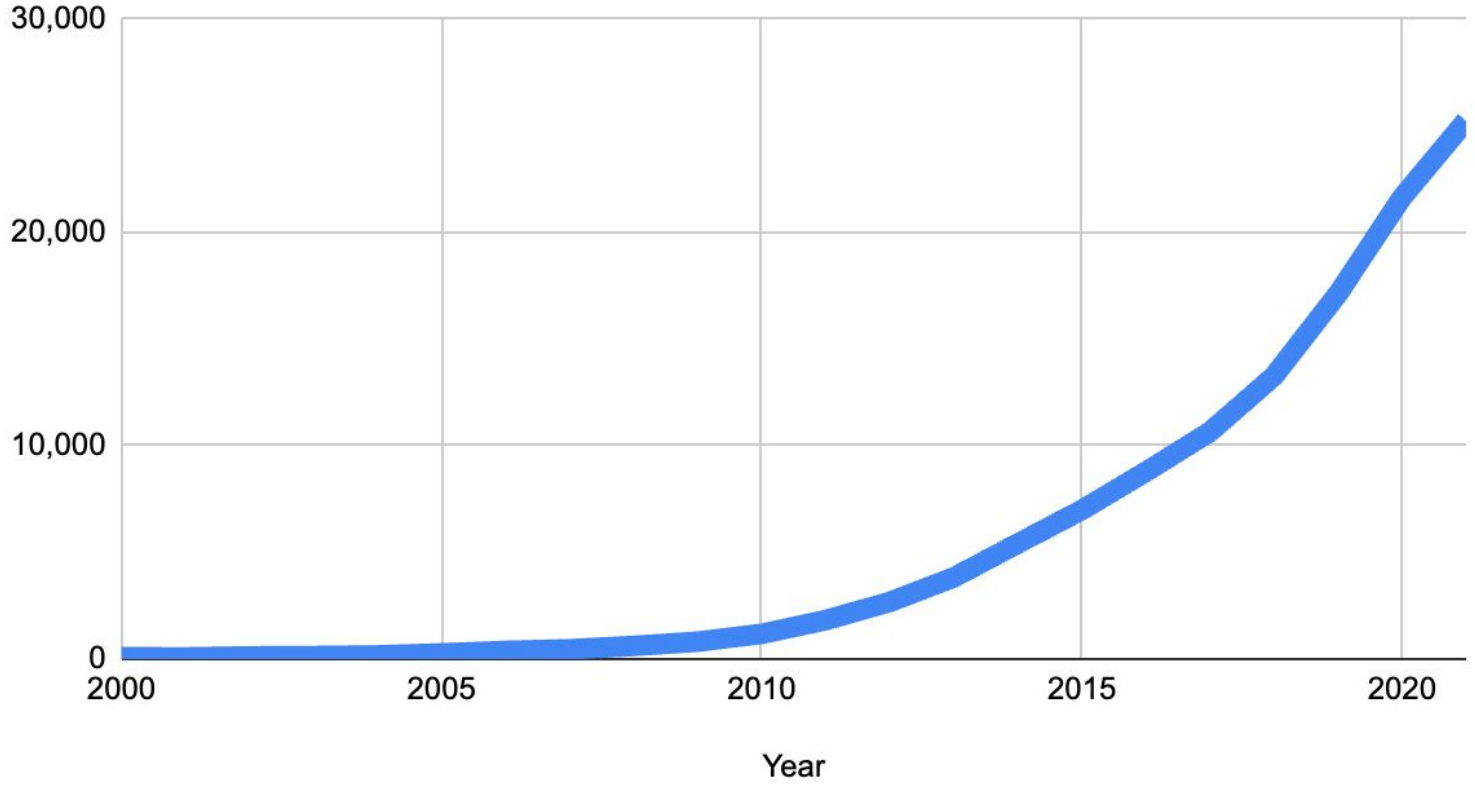
Christina O’Connor, RD

Christina is a registered dietitian and the Senior Healthcare Account Manager at Pendulum Therapeutics. She received her bachelor of science at James Madison University and completed her dietetic internship through the Virginia Department of Health.

Christina spent her early career working in pediatrics where she fell in love with microbiome science during her role as a NICU dietitian at The Children’s Hospital of the King’s Daughters. She was instrumental in helping to bring to life one of the area's first donor human milk banks.

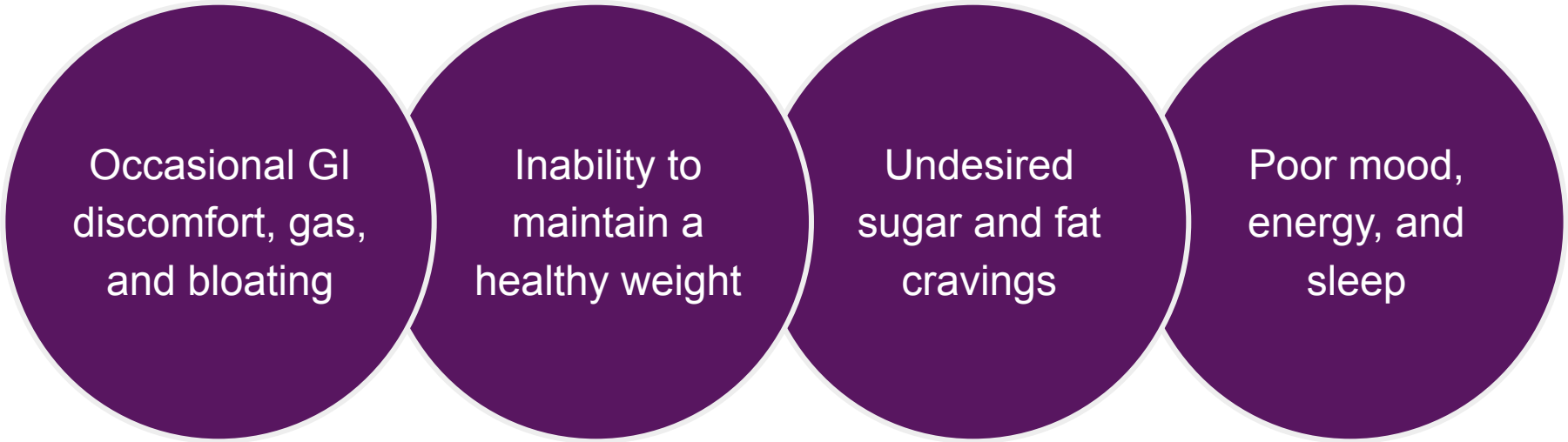


“Microbiome” Publications (Pubmed)



Gut Microbiome:
Impact on Health and Disease

Microbiome imbalances may present as:



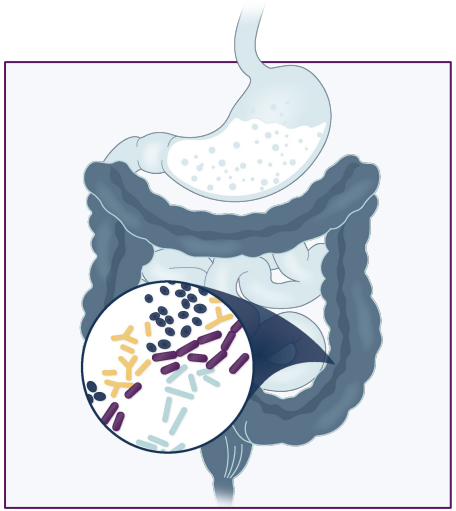
Science Behind Pendulum's Precision Strains

Cutting edge technology + microbiome science drives innovation for probiotics

Next-generation technology in needed to mass produce live anaerobic strains

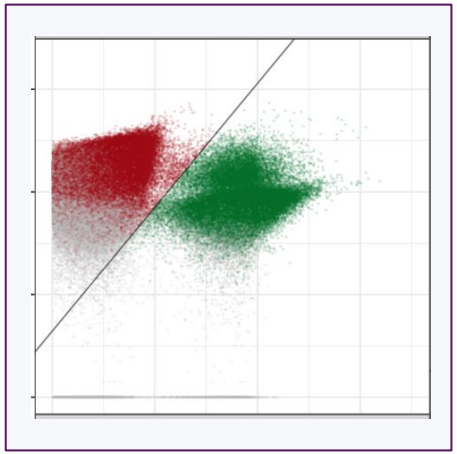
Next Generation Formulation

Gut microbiome
strict anaerobes

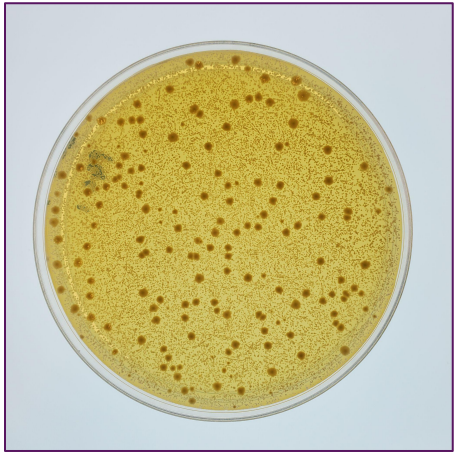


More accurate analysis

AFU
(Active Fluorescent Units)

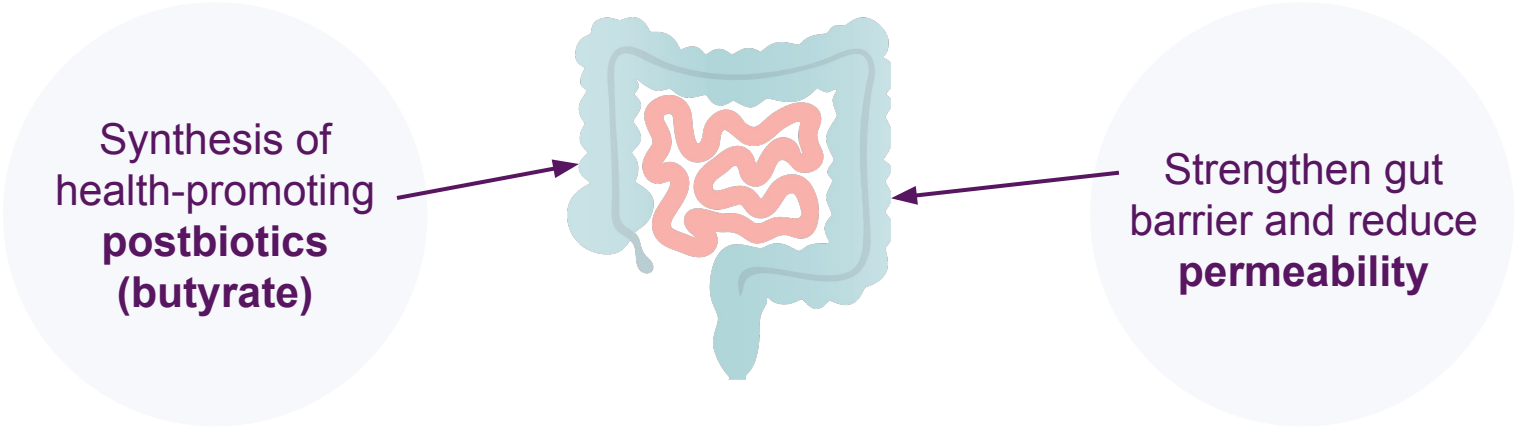


CFU
(Colony Forming Units)



Research shows that a healthy gut microbiome performs 2 key functions

Technological advancements in bacterial DNA sequencing allow scientists to identify precise probiotic strains that can perform these functions:



Trial design

12-week, balanced, parallel-arm, double-blind, placebo-controlled trial in type 2 diabetes

- Arms:
  - Placebo (excipients only)
  - WBF-010 (butyrate-producing)
  - WBF-011 (WBF-010 + Akkermansia + A.halli)

- Population:
  - 76 subjects across 6 study sites (intent-to-treat analysis)
  - 58 subjects analyzed (per protocol analysis)

- Key primary and secondary outcome measurements at week 12:
  - Safety & tolerability
  - Area under 3-hour glucose curves (ΔAUC) after meal Tolerance test (MTT)
  - Hemoglobin A1c (HbA1c)
  - C-reactive protein (CRP)

Open access Original research

BMJ Open Diabetes Research & Care

Improvements to postprandial glucose control in subjects with type 2 diabetes: a multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation

Fanny Perraudeau, Paul McMurdie, James Bullard, Andrew Cheng, Colleen Cutcliffe, Achal Deo, John Eid, Jessica Gines, Mohan Iyer, Nicholas Justice, Wesley T Loo, Madeleine Nemchek, Marcus Schicklberger, Michael Souza, Brendon Stoneburner, Surabhi Tyagi, Orville Kolterman

To cite: Perraudeau F, McMurdie P, Bullard J, *et al*. Improvements to postprandial glucose control in subjects with type 2 diabetes: a multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation. *BMJ Open Diab Res Care* 2020;**8**:e001319. doi:10.1136/bmjdrc-2020-001319

► Additional material is published online only. To view please visit the journal online (http://dx.doi.org/10.1136/bmjdrc-2020-001319).

FP and PM are joint first authors.

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Check for updates

Pendulum Therapeutics, Inc, San Francisco, California, USA

Correspondence to
Dr Orville Kolterman;
orville.kolterman@pendulum.co

ABSTRACT

Introduction A growing body of evidence suggests that specific, naturally occurring gut bacteria are underrepresented in the intestinal tracts of subjects with type 2 diabetes (T2D) and that their functions, like gut barrier stability and butyrate production, are important to glucose and insulin homeostasis. The objective of this study was to test the hypothesis that enteral exposure to microbes with these proposed functions can safely improve clinical measures of glycemic control and thereby play a role in the overall dietary management of diabetes.

Research design and methods We evaluated whether a probiotic comprised of these anaerobic bacteria would enhance dietary management by (1) manufacturing two novel probiotic formulations containing three (WBF-010) or five (WBF-011) distinct strains in a Current Good Manufacturing Practice (cGMP) facility, (2) establishing consistent live-cell concentrations, (3) confirming safety at target concentrations dispensed in both animal and human studies and (4) conducting a 12-week parallel, double-blind, placebo-controlled, proof-of-concept study in which subjects previously diagnosed with T2D (n=76) were randomly assigned to a two times a day regimen of placebo, WBF-010 or WBF-011.

Results No safety or tolerability issues were observed. Compared with the placebo group, subjects administered WBF-011 (which contains inulin, *Akkermansia muciniphila*, *Clostridium beijerinckii*, *Clostridium butyricum*, *Bifidobacterium infantis* and *Anaerobutyricum hallii*) significantly improved in the primary outcome, glucose total area under the curve (AUC; −36.1 mg/dL/180 min, p=0.0500 and also improved in secondary outcomes, glycated hemoglobin (A1c): −0.6, glucose incremental-AUC: −28.6 mg/dL/180 min.

Conclusions To our knowledge, this is the first randomized controlled trial to administer four of the five strains to human subjects with T2D. This proof-of-concept study (clinical trial number NCT03893422) shows that the intervention was safe and well tolerated and that supplementation with WBF-011 improves postprandial glucose control. The limited sample size and intersubject variability justifies future studies designed to confirm and expand on these observations.

Significance of this study

What is already known about this subject?
- Previous trials testing the efficacy of probiotics have used commercially available species that are not known to be absent or decreased in type 2 diabetes.
- Type 2 diabetes has been associated with abnormalities of the gut microbiome, particularly decreases in abundance of *Akkermansia muciniphila* and microbes involved in the conversion of dietary fiber to butyrate and other short-chain fatty acids.
- An increase in the abundance of butyrate producers and *Akkermansia muciniphila* induced by metformin treatment has been proposed as a supporting mechanism for the efficacy of this critical antidiabetic drug.

What are the new findings?
- Subjects with type 2 diabetes, currently using metformin, improved glucose control relative to placebo after 12-week administration of a novel probiotic formulation containing both butyrate producing bacteria and *Akkermansia muciniphila*.
- Improvement was observed via standard clinical measures for both short-term (meal-tolerance test glucose area under the curve) and long-term (A1c) glucose control, in a subject cohort that was stable on first-line treatment, predominantly metformin.
- No major probiotic supplementation-related side effects were observed.

INTRODUCTION

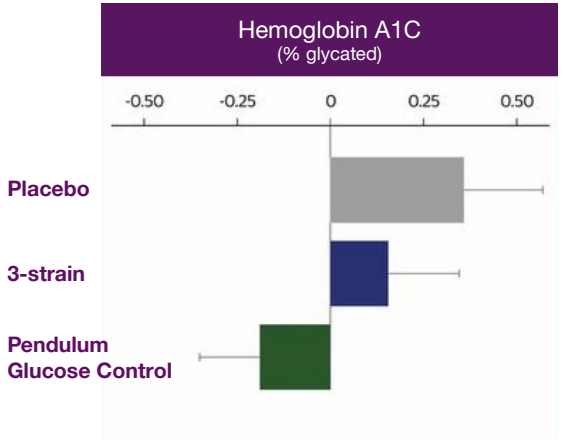
Diabetes is the seventh leading cause of death in the USA and in 2017 accounted for an estimated $327 billion in direct costs and lost productivity.[1 2] Over 20 million individuals are currently diagnosed with type 2 diabetes (T2D), some 90% of total diabetes diagnoses.[3] While genetic factors affect susceptibility,[4] it

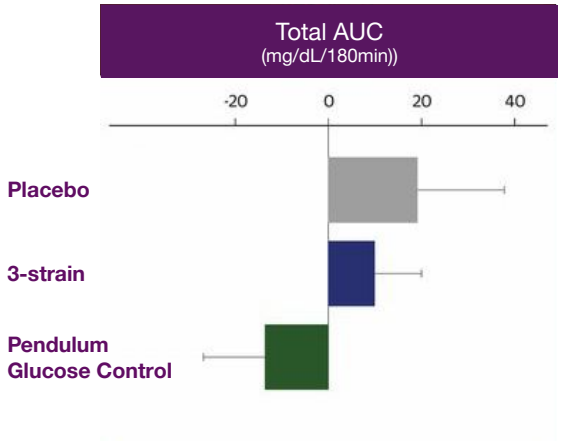
Improvements in postprandial glucose control and A1C in individuals with type 2 diabetes taking metformin

In a 12-week double-blinded, placebo controlled, randomized trial, Glucose Control, a probiotic formulation containing Akkermansia, was proven to:

- Lower postprandial glucose spikes by 33%
- Lower A1C by 0.6%
- Be safe and well-tolerated in study participants

Key takeaway: Administration of disease-relevant microbial species may be a promising new dietary management tool for improving glucose control in type 2 diabetes





Pendulum

Perraudeau F, McMurdie P, Bullard J, *et al.* Improvements to postprandial glucose control in subjects with type 2 diabetes: a multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulation. *BMJ Open Diabetes Research and Care* 2020;**8:**e001319. doi: 10.1136/bmjdrc-2020-001319

Learn more at PendulumLife.com

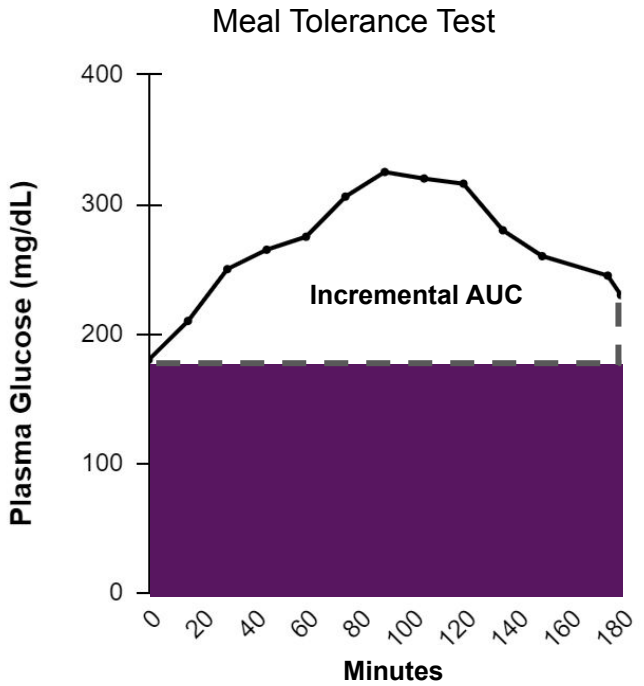
Study Design:

12-Week, balanced, parallel-arm, double-blind, placebo-controlled trial in early-stage Type 2 Diabetes

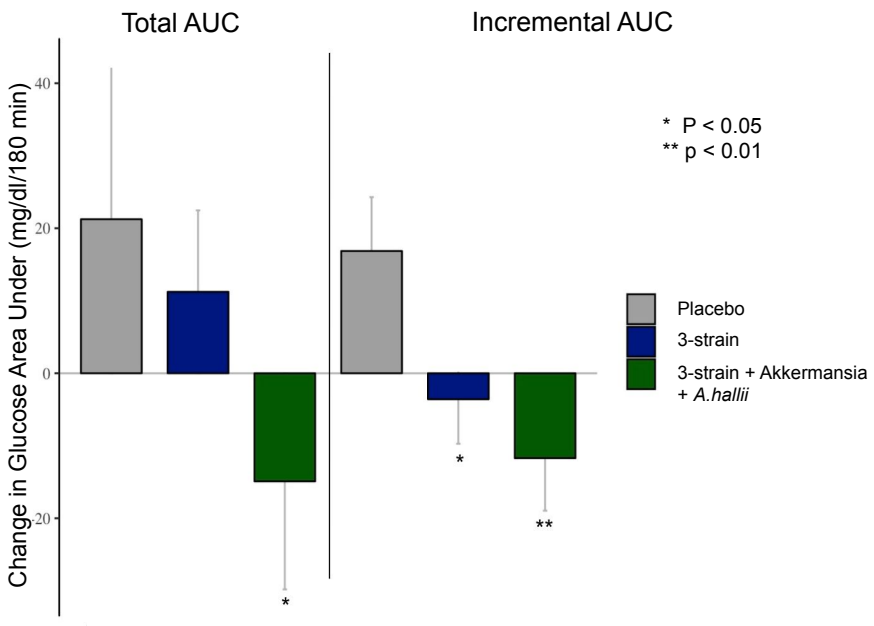
- Population:
  - 76 subjects across 6 study sites (intent-to-treat analysis)
  - 58 subjects analyzed (per protocol analysis)
- Arms:
  - Placebo = excipients only
  - WBF-010 = 3-strains
  - WBF-011 = 5-strains
- Key Primary and Secondary Outcome Measurements at Week 12:
  - Safety & Tolerability
  - Area under 3-hour glucose curves (ΔAUC) after Meal Tolerance Test (MTT)
  - Hemoglobin A1c (HbA1c)

Outcome: Improvement in Glucose Control

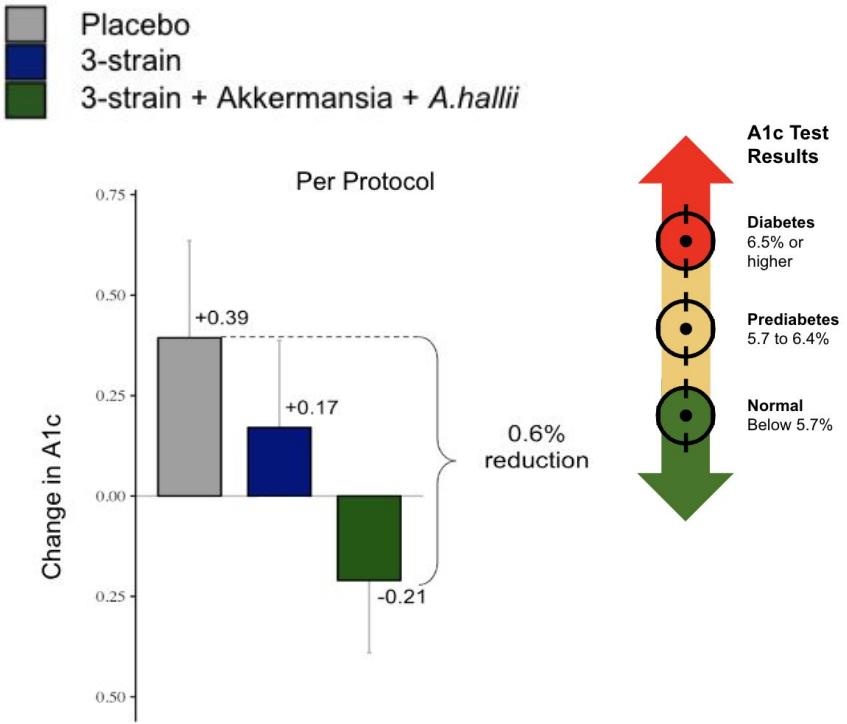
Defining Glucose AUC



Mixed Meal Tolerance Test



Outcome: Improvement in Glucose Control



	Pendulum Glucose Control	Metformin	SGLT2	GLP1	Insulin
A1C reduction	-0.6%	-0.7-1.2%	0.6-0.9%	-0.7-1.6%	-0.7-1.0%
AUC reduction	-33%	-20%	-23%	-50%	-32%
Product	Medical Probiotic	Drug	Drug	Drug (injection)	Drug (injection)
Side Effects	None known	GI distress Lactic Acidosis	Diabetic ketoacidosis Dehydration Genital infections	Pancreatitis Injection site reaction Nausea	Hypoglycemia Lipoatrophy ↑ CV risk Weight gain

Pendulum Glucose Control has efficacy for people with diabetes

Clinical Study:
Mechanism of Action

- Metabolite screening of samples from clinical trial in patients with type 2 diabetes.
- First publication demonstrating microbiome intervention can increase butyrate, UDCA and keystone strains
- Supports hypothesized mechanism of action of Pendulum Glucose Control
- 58 participants over 12 weeks
- Increased butyrate → increased secretion of GLP-1

McMurdie *et al. BMC Microbiology* (2022) 22:19
https://doi.org/10.1186/s12866-021-02415-8

BMC Microbiology

RESEARCH Open Access

Increased circulating butyrate and ursodeoxycholate during probiotic intervention in humans with type 2 diabetes

Paul J. McMurdie*, Magdalena K. Stoeva, Nicholas Justice, Madeleine Nemchek, Christian M. K. Sieber, Surabhi Tyagi, Jessica Gines, Connor T. Skennerton, Michael Souza, Orville Kolterman and John Eid

Abstract

Background: An increasing body of evidence implicates the resident gut microbiota as playing a critical role in type 2 diabetes (T2D) pathogenesis. We previously reported significant improvement in postprandial glucose control in human participants with T2D following 12-week administration of a 5-strain novel probiotic formulation ('WBF-011') in a double-blind, randomized, placebo controlled setting (NCT03893422). While the clinical endpoints were encouraging, additional exploratory measurements were needed in order to link the motivating mechanistic hypothesis - increased short-chain fatty acids - with markers of disease.

Results: Here we report targeted and untargeted metabolomic measurements on fasting plasma ($n = 104$) collected at baseline and end of intervention. Butyrate and ursodeoxycholate increased among participants randomized to WBF-011, along with compelling trends between butyrate and glycated haemoglobin (HbA1c). In vitro monoculture experiments demonstrated that the formulation's *C. butyricum* strain efficiently synthesizes ursodeoxycholate from the primary bile acid chenodeoxycholate during butyrogenic growth. Untargeted metabolomics also revealed coordinated decreases in intermediates of fatty acid oxidation and bilirubin, potential secondary signatures for metabolic improvement. Finally, improvement in HbA1c was limited almost entirely to participants not using sulfonylurea drugs. We show that these drugs can inhibit growth of formulation strains in vitro.

Conclusion: To our knowledge, this is the first description of an increase in circulating butyrate or ursodeoxycholate following a probiotic intervention in humans with T2D, adding support for the possibility of a targeted microbiome-based approach to assist in the management of T2D. The efficient synthesis of UDCA by *C. butyricum* is also likely of interest to investigators of its use as a probiotic in other disease settings. The potential for inhibitory interaction between sulfonylurea drugs and gut microbiota should be considered carefully in the design of future studies.

Keywords: *Anaerobutyricum hallii*, *Akkermansia muciniphila*, Bile acids, Butyrate, *Clostridium butyricum*, Metabolomics, Short-chain fatty acids, Sulfonylurea, Type 2 diabetes, Ursodeoxycholate

Background

34.2 million Americans were estimated to have diabetes in 2018 -- over one in ten -- with an estimated annual cost burden of $327 billion and growing, due to an estimated 1.5 million new diagnoses annually [1]. Approximately 90% of total diabetes diagnoses are type 2 (T2D). While genetic factors affect susceptibility, it is increasingly evident that Western lifestyle and diet plays a large role in

*Correspondence: paul.mcmurdie@pendulum.co
Pendulum Therapeutics, Inc, 933 20th Street, San Francisco, CA 94107, USA

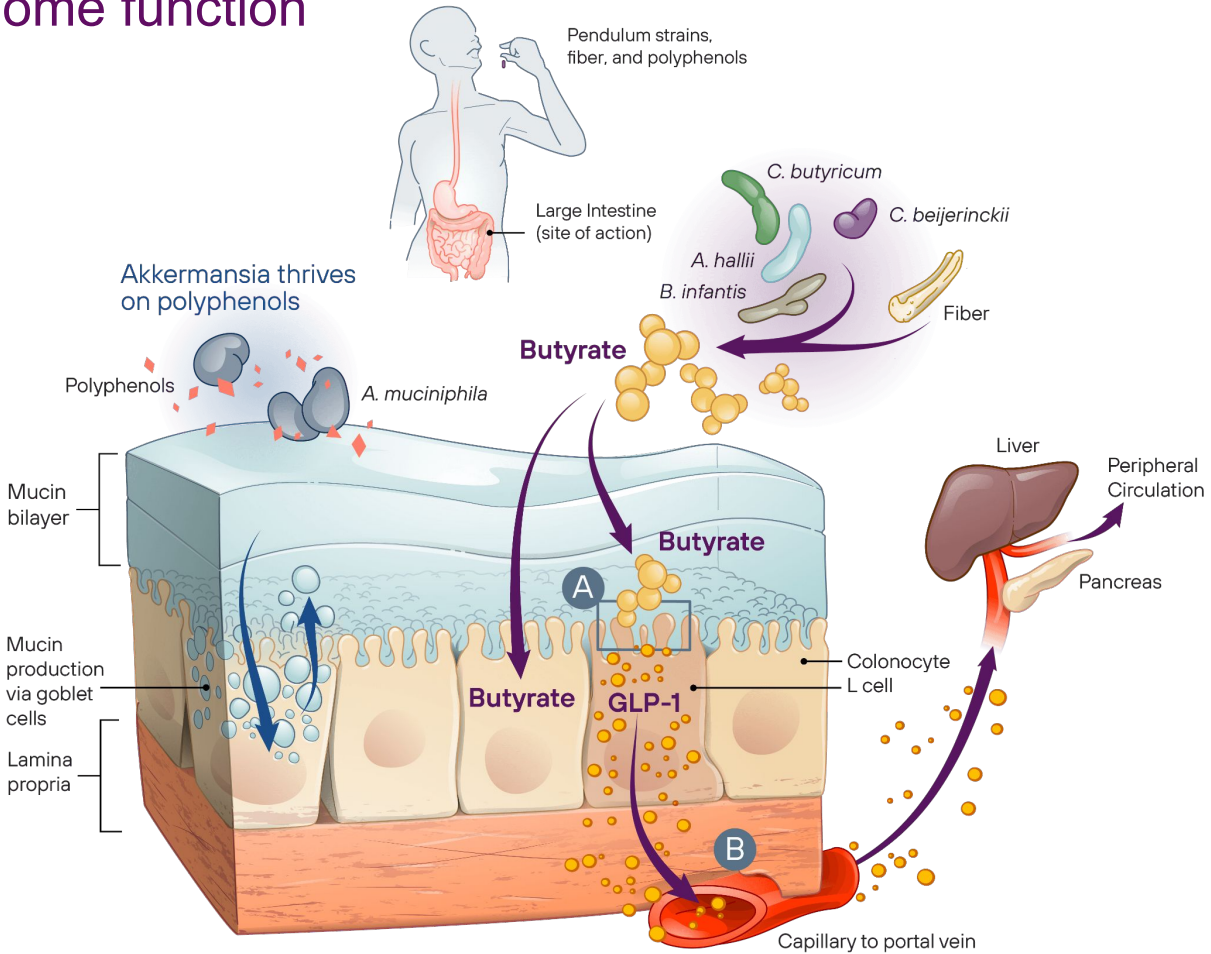
Overview of gut microbiome function

Pendulum Strains

Mucin fortifier
Akkermansia muciniphila

Butyrate producers
Clostridium butyricum
Clostridium beijerinckii
Anaerobutyricum hallii

Primary fermenter
Bifidobacterium infantis



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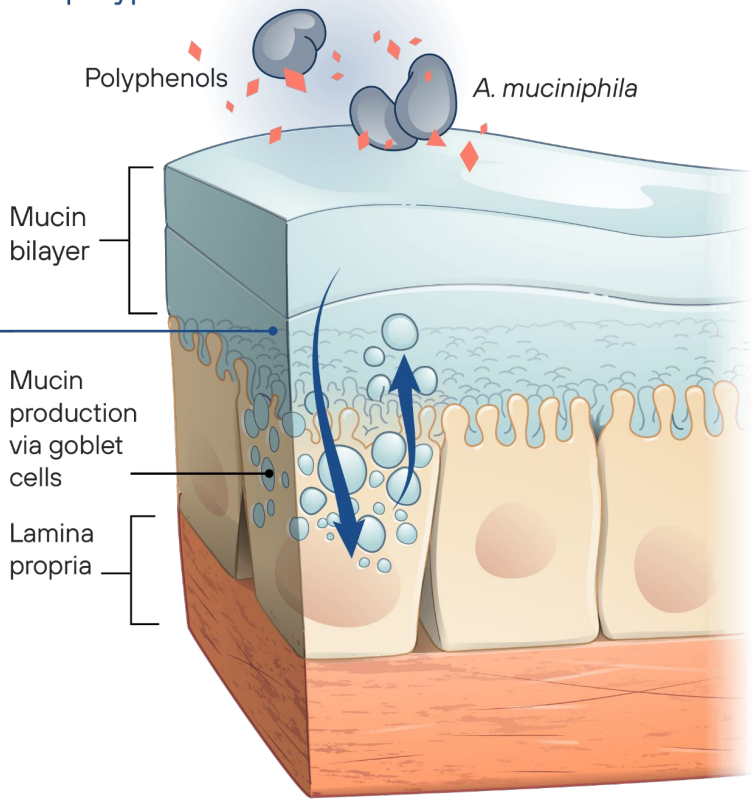
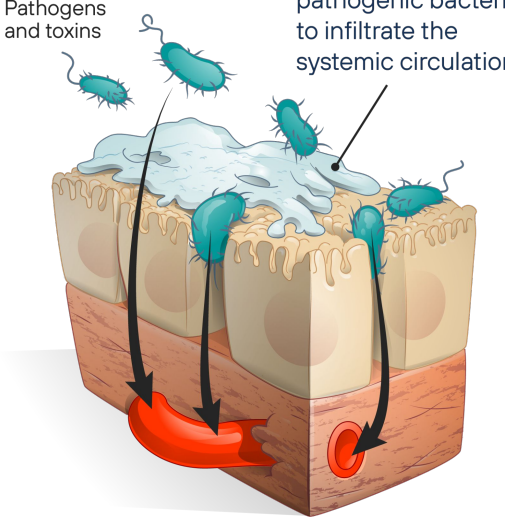
Akkermansia thrives on polyphenols



↑ Mucin production

↑ Barrier function
↑ Gut immunity homeostasis



Thinning mucin layer can lead to the development of gut dysbiosis, allowing pathogenic bacteria to infiltrate the systemic circulation.

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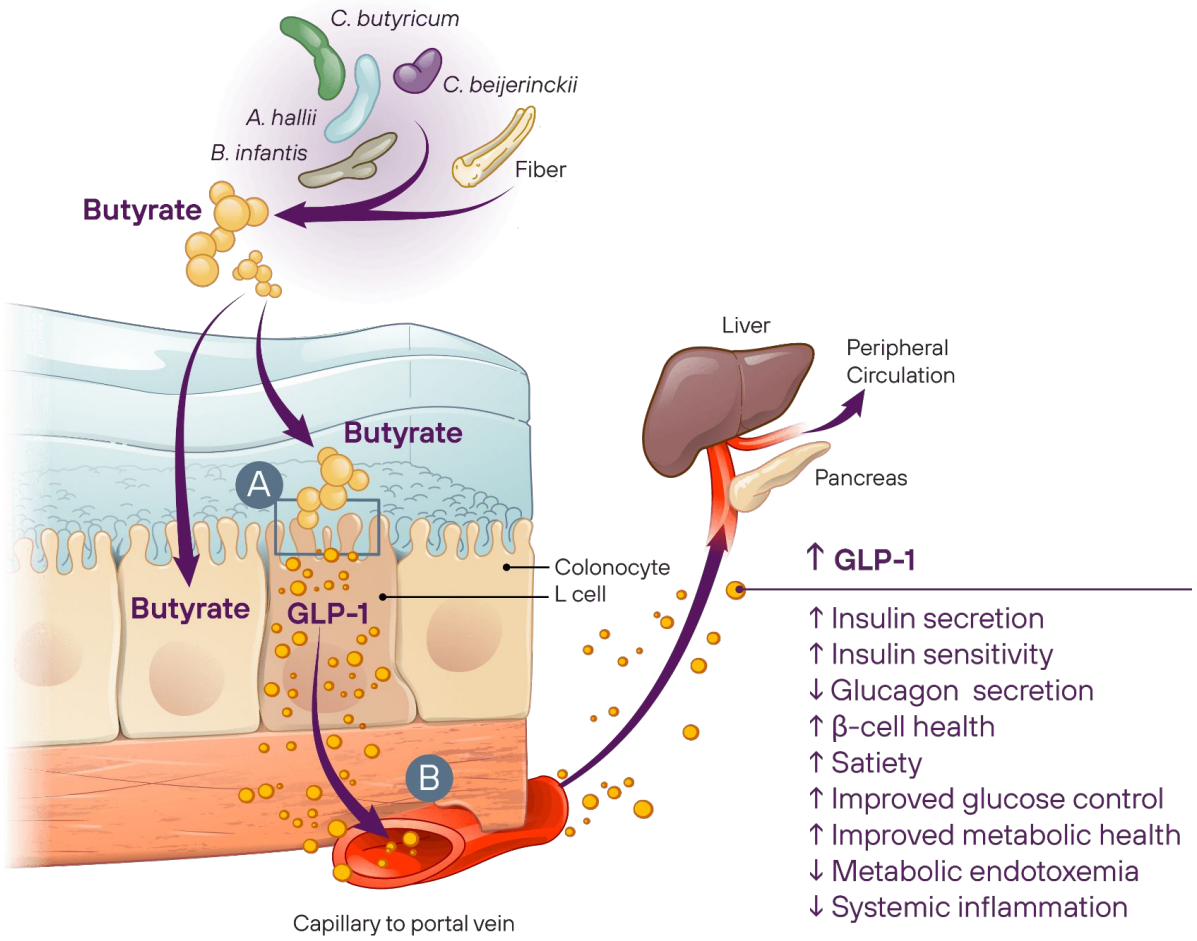
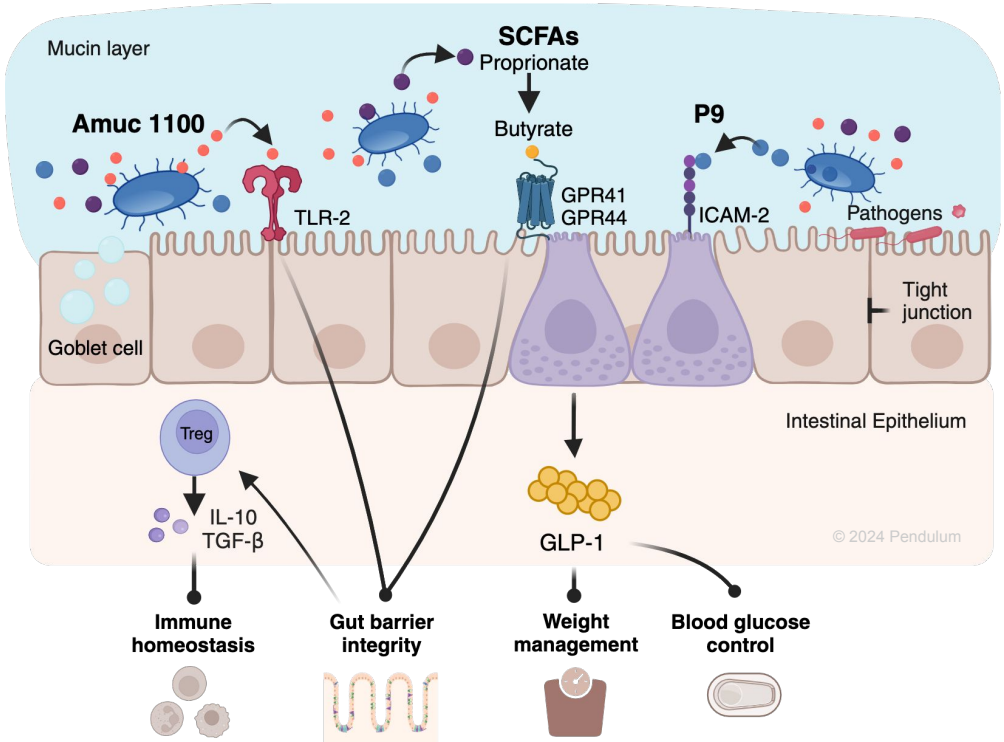

C. butyricum
C. beijerinckii
A. hallii
B. infantis
Fiber
Butyrate
Butyrate
A
Butyrate
GLP-1
Colonocyte
L cell
B
Capillary to portal vein
Liver
Peripheral Circulation
Pancreas
↑ GLP-1
↑ Insulin secretion
↑ Insulin sensitivity
↓ Glucagon secretion
↑ β-cell health
↑ Satiety
↑ Improved glucose control
↑ Improved metabolic health
↓ Metabolic endotoxemia
↓ Systemic inflammation

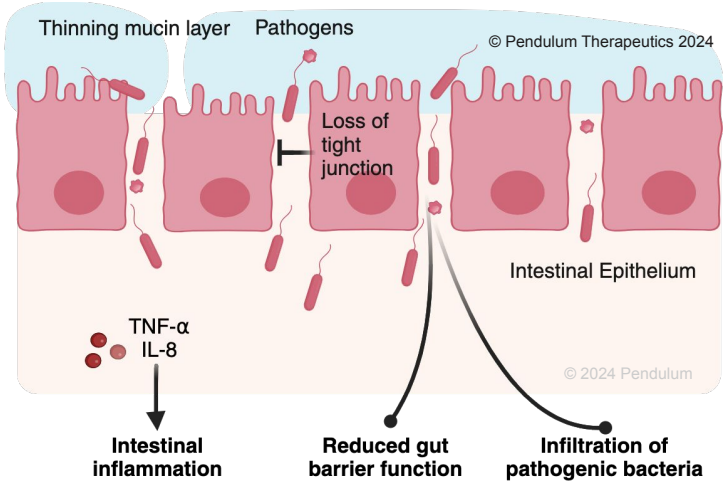
Pendulum

Known *Akkermansia* benefits and mechanism today

Akkermansia

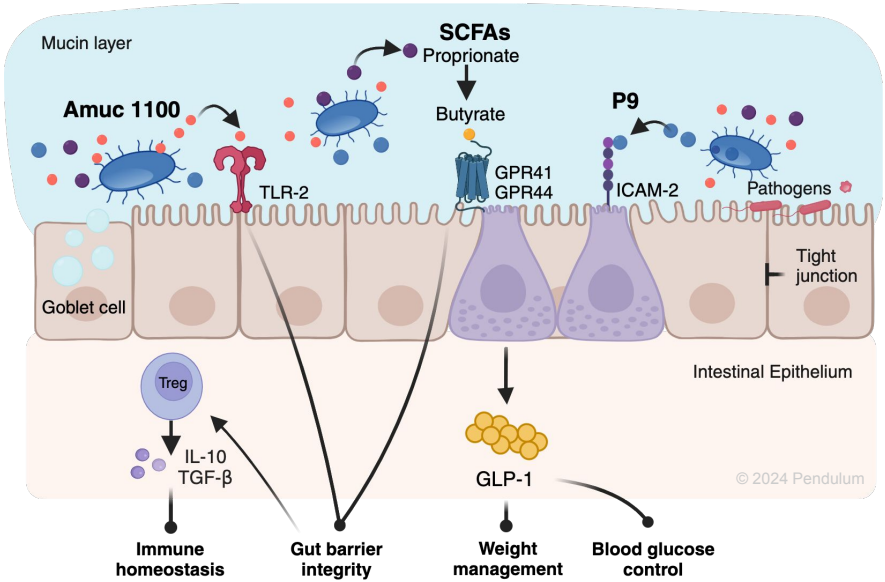


No *Akkermansia*

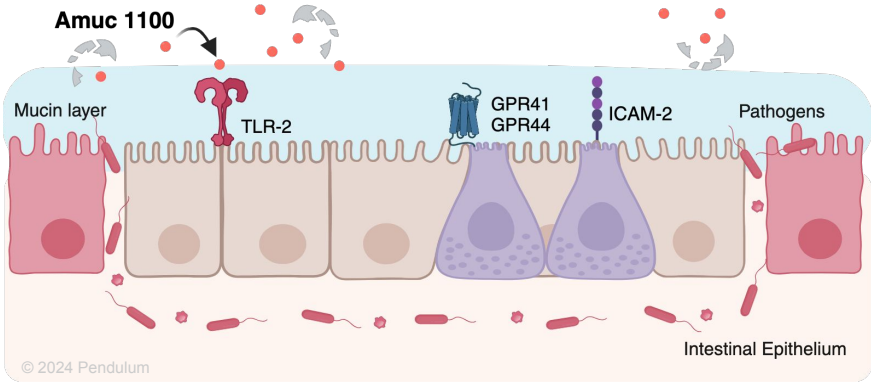


Live vs pasteurized *Akkermansia*: mucin regulation

Live *Akkermansia*



Pasteurized *Akkermansia*



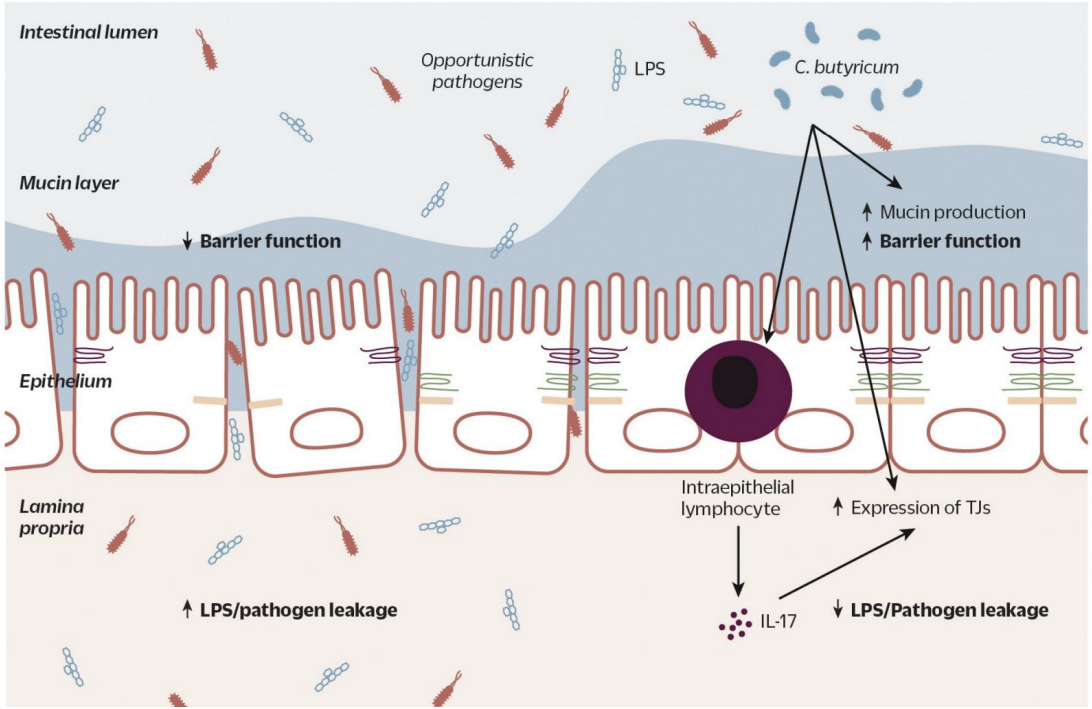
Only live *Akkermansia* can interact with and regulate the mucin layer

- Live *Akkermansia* consumes mucin and stimulates goblet cells to produce new mucin
- Supplementing with pasteurized bacterial cells does not enable this important process for maintaining gut barrier integrity

Clostridium butyricum strengthens the gut barrier

Key takeaways:

- Multiple studies note increases in fecal butyrate concentrations following *C. butyricum* supplementation and/or the proliferation of other butyrate-producing bacteria.
- *C. butyricum* strains improve gut barrier function by increasing the thickness of the mucosal layer and increasing expression of tight junction proteins.



Stoeva, Magdalena K et al. "Butyrate-producing human gut symbiont, *Clostridium butyricum*, and its role in health and disease." *Gut microbes* vol. 13,1 (2021): 1-28. doi:10.1080/19490976.2021.1907272

Akkermansia is associated with reduced risk of obesity

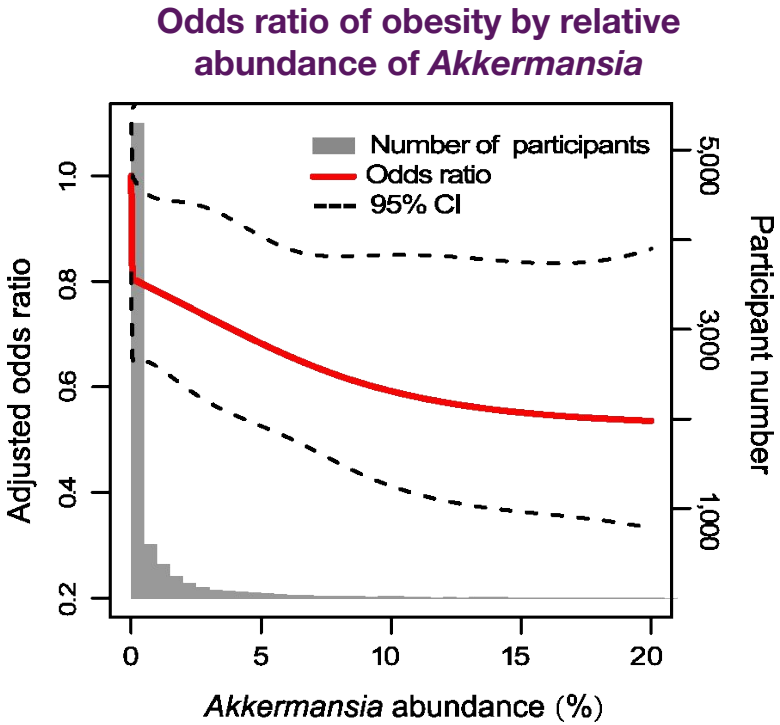
American Gut Project
10,534 participants
20 to 99 years of age.

Outcomes:

- The BMI and [Akkermansia] inversely correlated (across the entire study population; $p < 0.001$)
- Modeling showed: 10% higher *Akkermansia* reduces risk for obesity approximately 26%, on average

Key takeaway:

High relative abundance of *Akkermansia* is associated with low risk of obesity independent of age, sex, smoking, alcohol drinking, diet and country.



Zhou, Q., Zhang, Y., Wang, X. *et al.* Gut bacteria *Akkermansia* is associated with reduced risk of obesity: evidence from the American Gut Project. *Nutr Metab (Lond)* 17, 90 (2020). Link to creative commons license http://creativecommons.org/licenses/by/4.0/.â

GLP-1 Probiotic Consumer Survey 6 Week Results

Pendulum Research Program

- 3 Month Consumer Study
- November 2023 — February 2024
  - 274 Participants - 6 week analysis
  - Analysis ongoing - 3 month results

- Recruitment
  - screening: 18+, live in US, willing

Food Cravings Measured By

- Validated Questionnaire
  - Food Cravings Inventory (FCI-II)+

Taking Pendulum GLP-1 Probiotic Containing

  - Akkermansia muciniphila
  - Clostridium butyricum
  - Bifidobacterium infantis

A craving is defined as an intense desire to consume a particular food (or food type) that is difficult to resist.

Over the past month, or since the last time you completed this inventory, how often have you experienced a craving for the food?

A= Never
B= Rarely (once or twice)
C=Sometimes
D=Often
E=Always/almost every day

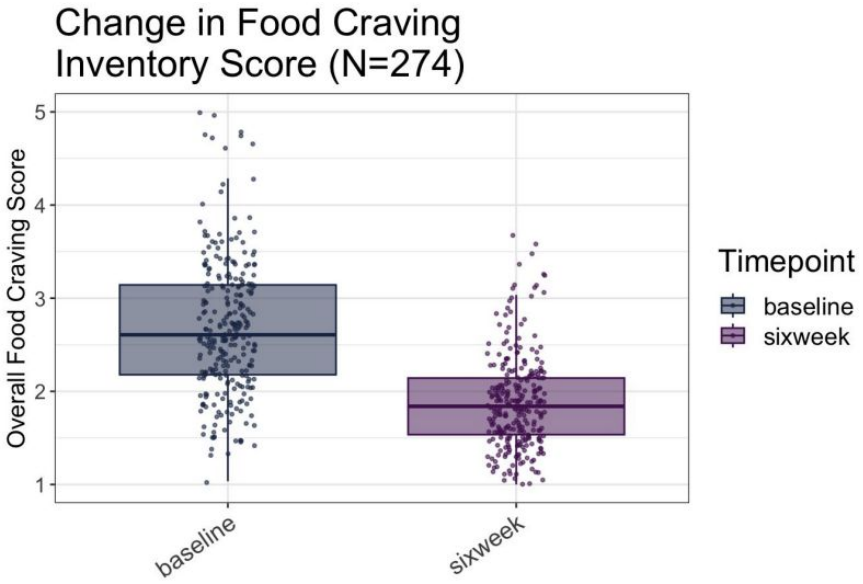
	Never	Rarely	Sometimes	Often	Always	Factor loading
1. Cake	A	B	C	D	E	Sweets
2. Pizza	A	B	C	D	E	Fast food fat
3. Fried Chicken	A	B	C	D	E	High fat
4. Gravy	A	B	C	D	E	High fat
5. Sandwich Bread	A	B	C	D	E	Carb/starch
6. Sausage	A	B	C	D	E	High fat
7. French fries	A	B	C	D	E	Fast food fat
8. Cinnamon Rolls	A	B	C	D	E	Sweets
9. Rice	A	B	C	D	E	Carb/starch
10. Hot dog	A	B	C	D	E	High fat
11. Hamburger	A	B	C	D	E	Fat food fat
12. Biscuits	A	B	C	D	E	Carb/starch
13. Ice cream	A	B	C	D	E	Sweets
14. Pasta	A	B	C	D	E	Carb/starch
15. Fried fish	A	B	C	D	E	High fat
16. Cookies	A	B	C	D	E	Sweets
17. Chocolate	A	B	C	D	E	Sweets
18. Pancakes or waffles	A	B	C	D	E	Carb/starch
19. Corn bread	A	B	C	D	E	High fat
20. Chips	A	B	C	D	E	Fast food fat
21. Rolls	A	B	C	D	E	Carb/starch
22. Cereal	A	B	C	D	E	Carb/starch
23. Donuts	A	B	C	D	E	Sweets
24. Candy	A	B	C	D	E	Sweets
25. Brownies	A	B	C	D	E	Sweets
26. Bacon	A	B	C	D	E	High fat
27. Steak	A	B	C	D	E	High fat
28. Baked potato	A	B	C	D	E	Carb/starch

+White MA, Whisenhunt BL, Williamson DA, Greenway FL, Netemeyer RG. Development and validation of the food-craving inventory. Obes Res. 2002 Feb;10(2):107-14. doi: 10.1038/oby.2002.17. PMID: 11836456.

Total food cravings were reduced with 6 weeks of GLP-1 Probiotic supplementation*

Key takeaway:

91% of participants experienced reduced food cravings



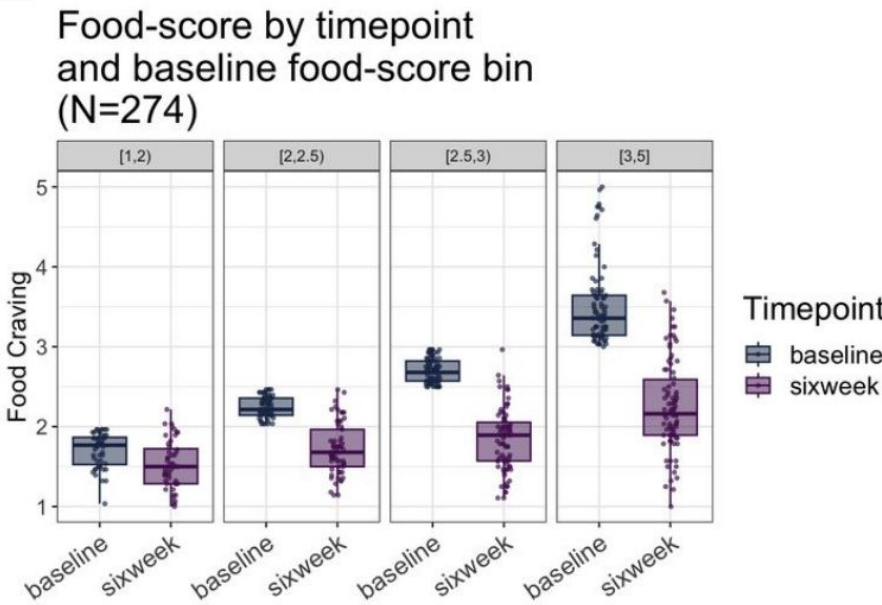
*p value < 0.001

*based on a survey of 274 people

Total food cravings were reduced with 6 weeks of GLP-1 Probiotic supplementation*

Key takeaway:

Higher total cravings at baseline experienced greater reduction of cravings at 6 weeks



% Participants that experienced improvements, by baseline overall food craving bins:

65% 90% 99% 98%

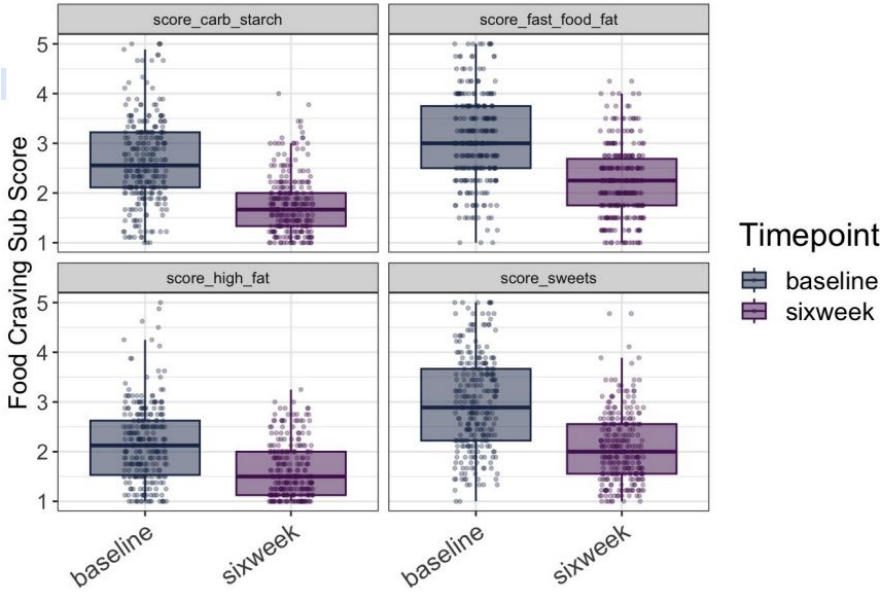
*p value < 0.001

*based on a survey of 274 people

Total food cravings were reduced with 6 weeks of GLP-1 Probiotic supplementation*

Participants experienced improvements in food cravings across all 4 categories tested:

- 88% - Sweets
- 87% - Carbs/Starches
- 85% - Fast Food Fats
- 82% - High Fats



*based on a survey of 274 people

Impact of a GLP-1 probiotic on food cravings

97% of healthcare providers recommend as a way to reduce food cravings

*based on a survey of 64 healthcare providers

"This has really helped provide our patients a more **smooth transition off of GLP-1 medications**. Once they completely stop the medication, **the food cravings have been far less** than when they start taking a GLP-1 probiotic for 90 days before they end the program. It has been a big help to our practice and for our patients."

Impact of three-strain probiotic formulation on food cravings

In a survey of healthcare providers who use a
proprietary three-strain probiotic formulation containing live Akkermansia in their practice:

78%	Recommend as a **strategy to help patients transition off of GLP-1 medications**, specifically to manage food cravings associated with this transition
64%	Recommend as a **first line approach before prescribing a GLP-1 medication**
53%	Recommend **in combination with GLP-1 medications**

*based on a survey of 64 healthcare providers

Impact of a GLP-1 Probiotic on food cravings

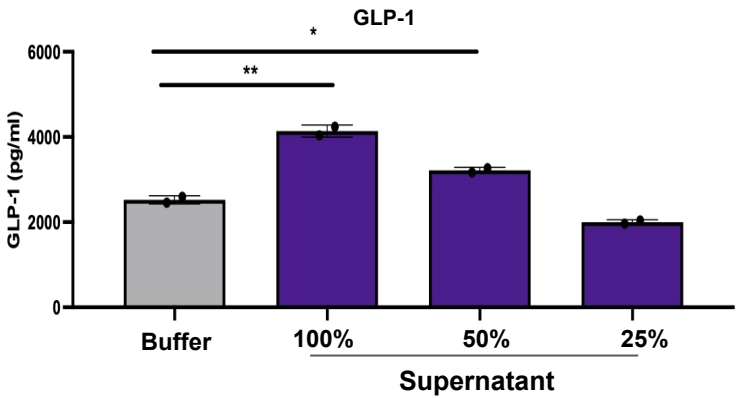
In a survey of healthcare providers who use a
a GLP-1 probiotic in their practice:

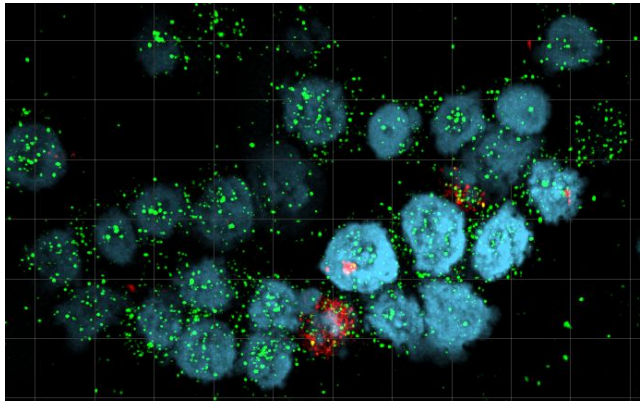
78%	Recommend as a **strategy to help patients transition off of GLP-1 medications**, specifically to manage food cravings associated with this transition
64%	Recommend as a **first line approach before prescribing a GLP-1 medication**
53%	Recommend **in combination with GLP-1 medications**

*based on a survey of 64 healthcare providers

Akkermansia plays a role in boosting GLP-1 production

Akkermansia supernatant stimulates human enteric cells to secrete GLP-1 and PYY





DAPI
GLP-1
PYY

Data on file.

Key Takeaways

In a clinical trial, Pendulum strains significantly lowered postprandial glucose spikes by 33% and A1c by 0.6 percentage points

Pendulum strains are shown to increase butyrate, UDCA, and *Akkermansia muciniphila*, all of which lead to increased GLP-1

Polyphenols can act as prebiotics for certain bacterial strains, specifically *Akkermansia muciniphila*

Administration of targeted probiotic species may be a promising new dietary management tool for improving health outcomes

Butyricum improves gut barrier function by increasing the thickness of the mucosal layer and increasing expression of tight junction proteins

Pendulum Products

Glucose Control

Strain Information:

- *Akkermansia muciniphila WB-STR-0001*
- *Anaerobutyricum hallii WB-STR-0008*
- *Clostridium butyricum* WB-STR-0006
- *Clostridium beijerinckii WB-STR-0005*
- *Bifidobacterium infantis 100*
    - AFU Information: 2 billion AFUs per dose

Attributes:

- Clinically proven to improve A1C and postprandial blood glucose spikes in people with type 2 diabetes taking metformin
- Proprietary blend of novel anaerobic strains

Metabolic Daily

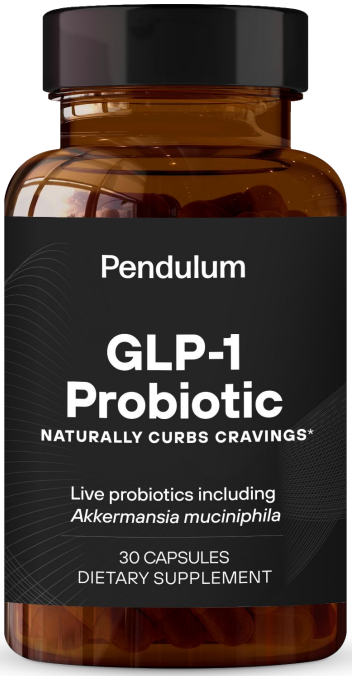
Strain Information:

- *Akkermansia muciniphila WB-STR-0001*
- *Anaerobutyricum hallii WB-STR-0008*
- *Clostridium butyricum* WB-STR-0006
- *Clostridium beijerinckii WB-STR-0005*
- *Bifidobacterium infantis 100*
    - AFU Information: 300 million AFUs per capsule

Attributes:

- Reduces sugar cravings and energy slumps
- Boosts sugar and carbohydrate metabolism
- Increases butyrate and GLP-1
- Supports a healthy weight

GLP-1 Probiotic



Strain Information:

- *Clostridium butyricum* WB-STR-0006
- *Akkermansia muciniphila WB-STR-0001*
- *Bifidobacterium infantis 100*
    - AFU Information: 500 million AFUs per capsule

Attributes:

- Enhances natural GLP-1 production*
- Helps maintain a healthy weight
- 91% reported reduced overall food cravings**

*based on preclinical studies
**based on a survey of 274 people

Pendulum Akkermansia

Strain Information:

- *Akkermansia muciniphila* WB-STR-0001
  - AFU Information: 100 million AFUs per capsule

Attributes:

- The first new probiotic strain to enter the market in 50 years
- Keystone strain for strengthening gut lining
- 2000+ scientific publications

Polyphenol Booster

Product Information:

- Pomegranate Extract (90% Ellagic acid)
- Grape Seed Extract (95% Proanthocyanidins)
- Green Tea Extract (95% EGCG)

Attributes:

- Boosts Akkermansia levels in the gut
- Supports healthy aging
- Increases antioxidants and polyphenols
- Supports cardio health

Butyricum

Strain Information:

- *Clostridium butyricum* WB-STR-0006
  - AFU Information: 15 million AFUs per capsule

Attributes:

- Helps relieve occasional gas, bloating, diarrhea and constipation
- Developed in partnership with the world's leading GI researchers
- Stimulates butyrate production

Dietary Polyphenols Increase *Akkermansia* to Combat Metabolic Syndrome

- Daily oral administration of cranberry extract for 8 weeks prevented weight gain and ameliorated several features of metabolic syndrome in association with a strong increase in the abundance of *Akkermansia* in the gut microbiota of mice.[1]

- Healthy volunteers drinking a pomegranate extract displayed increased presence of *Akkermansia* in stool samples.[2]

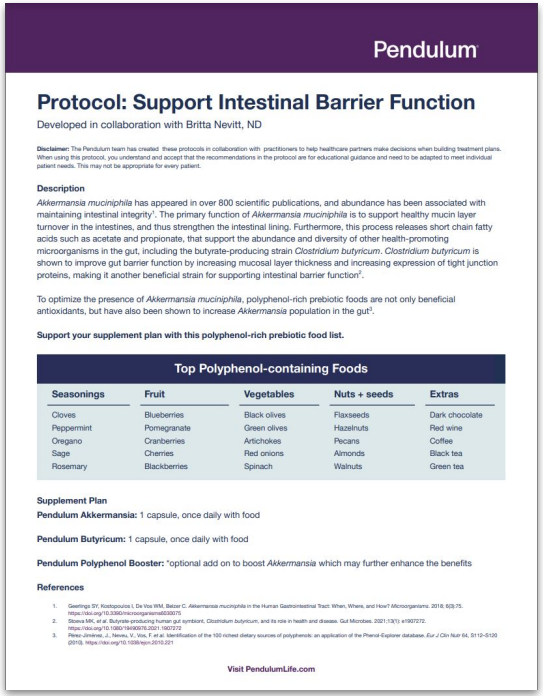
- Administration of *Akkermansia* as a probiotic was shown to alleviate obesity-related metabolic disturbances while increasing Muc2 production in mice, thus improving mucus layer thickness and intestinal barrier.[3]

1. Anh^e FF, et al. A polyphenol-rich cranberry extract protects from diet-induced obesity, insulin resistance and intestinal inflammation in association with increased Akkermansia spp. population in the gut microbiota of mice. Gut 2014; 64:872-83
2. Li Z, et al. Pomegranate extract induces ellagitannin metabolite formation and changes stool microbiota in healthy volunteers. Food Funct 2015; 6:2487-95
3. Everard A, et al. Cross-talk between akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A 2013; 110:9066-71

Pendulum

Pendulum Protocols

Support Intestinal Barrier Function



Pendulum

Protocol: Support Intestinal Barrier Function

Developed in collaboration with Britta Nevitt, ND

Disclaimer: The Pendulum team has created these protocols in collaboration with practitioners to help healthcare partners make decisions when building treatment plans. When using this protocol, you understand and accept that the recommendations in the protocol are for educational guidance and need to be adapted to meet individual patient needs. This may not be appropriate for every patient.

Description

Akkermansia muciniphila has appeared in over 800 scientific publications, and abundance has been associated with maintaining intestinal integrity[1]. The primary function of *Akkermansia muciniphila* is to support healthy mucin layer turnover in the intestines, and thus strengthen the intestinal lining. Furthermore, this process releases short chain fatty acids such as acetate and propionate, that support the abundance and diversity of other health-promoting microorganisms in the gut, including the butyrate-producing strain *Clostridium butyricum*. *Clostridium butyricum* is shown to improve gut barrier function by increasing mucosal layer thickness and increasing expression of tight junction proteins, making it another beneficial strain for supporting intestinal barrier function[2].

To optimize the presence of *Akkermansia muciniphila*, polyphenol-rich prebiotic foods are not only beneficial antioxidants, but have also been shown to increase *Akkermansia* population in the gut[3].

Support your supplement plan with this polyphenol-rich prebiotic food list.

Top Polyphenol-containing Foods

Seasonings	Fruit	Vegetables	Nuts + seeds	Extras
Cloves	Blueberries	Black olives	Flaxseeds	Dark chocolate
Peppermint	Pomegranate	Green olives	Hazelnuts	Red wine
Oregano	Cranberries	Artichokes	Pecans	Coffee
Sage	Cherries	Red onions	Almonds	Black tea
Rosemary	Blackberries	Spinach	Walnuts	Green tea

Supplement Plan

Pendulum Akkermansia: 1 capsule, once daily with food

Pendulum Butyricum: 1 capsule, once daily with food

Pendulum Polyphenol Booster: *optional add on to boost *Akkermansia* which may further enhance the benefits

References

1. Geerlings SY, Kostopoulos I, De Vos WM, Belzer C. *Akkermansia muciniphila* in the Human Gastrointestinal Tract: When, Where, and How? *Microorganisms*. 2018; 6(3):75. https://doi.org/10.3390/microorganisms6030075
2. Stowa MK, et al. Butyrate-producing human gut symbiont, *Clostridium butyricum*, and its role in health and disease. Gut Microbes. 2021;13(1): e1907272. https://doi.org/10.1080/19490976.2021.1907272
3. Pérez-Jiménez, J., Neveu, V., Vos, F. et al. Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *Eur J Clin Nutr* 64, S1:12-S1:00 (2010). https://doi.org/10.1038/ejcn.2010.221

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Why is this important?

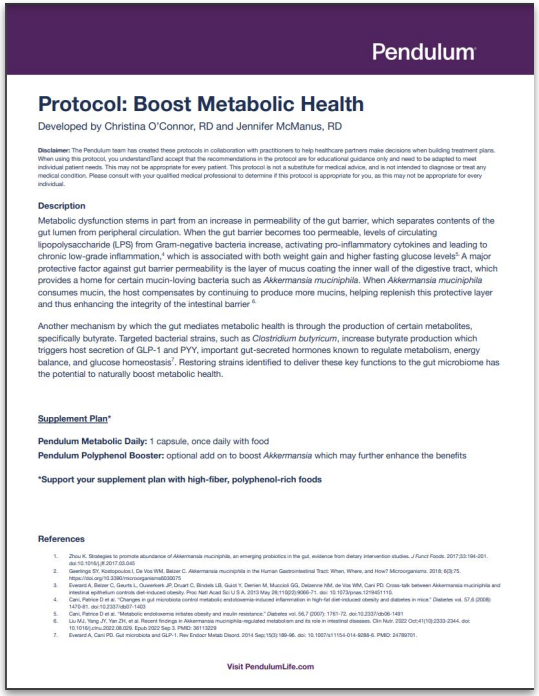
- The primary function of ***Akkermansia muciniphila*** is to support healthy mucin layer turnover in the intestines, and thus strengthen the intestinal lining
- ***Clostridium butyricum*** is shown to improve gut barrier function by increasing mucosal layer thickness and increasing expression of tight junction proteins

Supplement Plan

- Pendulum Akkermansia: 1 capsule, once daily with food
- Pendulum Butyricum: 1 capsule, once daily with food
- Pendulum Polyphenol Booster: *optional add on to boost Akkermansia which may further enhance the benefits

*Support your supplement plan with high-fiber, polyphenol-rich foods

Boost Metabolic Health



Pendulum

Protocol: Boost Metabolic Health

Developed by Christina O'Connor, RD and Jennifer McManus, RD

Disclaimer: The Pendulum team has created these protocols in collaboration with practitioners to help healthcare partners make decisions when building treatment plans. When using this protocol, you understandTand accept that the recommendations in the protocol are for educational guidance only and need to be adapted to meet individual patient needs. This may not be appropriate for every patient. This protocol is not a substitute for medical advice, and is not intended to diagnose or treat any medical condition. Please consult with your qualified medical professional to determine if this protocol is appropriate for you, as this may not be appropriate for every individual.

Description

Metabolic dysfunction stems in part from an increase in permeability of the gut barrier, which separates contents of the gut lumen from peripheral circulation. When the gut barrier becomes too permeable, levels of circulating lipopolysaccharide (LPS) from Gram-negative bacteria increase, activating pro-inflammatory cytokines and leading to chronic low-grade inflammation,[4] which is associated with both weight gain and higher fasting glucose levels[5] A major protective factor against gut barrier permeability is the layer of mucus coating the inner wall of the digestive tract, which provides a home for certain mucin-loving bacteria such as *Akkermansia muciniphila*. When *Akkermansia muciniphila* consumes mucin, the host compensates by continuing to produce more mucins, helping replenish this protective layer and thus enhancing the integrity of the intestinal barrier.[6]

Another mechanism by which the gut mediates metabolic health is through the production of certain metabolites, specifically butyrate. Targeted bacterial strains, such as *Clostridium butyricum*, increase butyrate production which triggers host secretion of GLP-1 and PYY, important gut-secreted hormones known to regulate metabolism, energy balance, and glucose homeostasis[7]. Restoring strains identified to deliver these key functions to the gut microbiome has the potential to naturally boost metabolic health.

Supplement Plan*

Pendulum Metabolic Daily: 1 capsule, once daily with food

Pendulum Polyphenol Booster: optional add on to boost *Akkermansia* which may further enhance the benefits

***Support your supplement plan with high-fiber, polyphenol-rich foods**

References

1. Zhou K. Strategies to promote abundance of Akkermansia muciniphila, an emerging probiotics in the gut, evidence from dietary intervention studies. *J Funct Foods*. 2017;33:194-201. doi:10.1016/j.jff.2017.03.045
2. Geerlings SY, Kostopoulos I, De Vos WM, Belzer C. Akkermansia muciniphila in the Human Gastrointestinal Tract: When, Where, and How? Microorganisms. 2018; 6(3):75. https://doi.org/10.3390/microorganisms6030075
3. Everard A, Belzer C, Geurts L, Ouwerkerk JP, Druart C, Bindels LB, Guiot Y, Derrien M, Muccioli GG, Delzenne NM, de Vos WM, Cani PD. Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proc Natl Acad Sci U S A. 2013 May 28;110(22):9066-71. doi: 10.1073/pnas.1219451110.
4. Cani, Patrice D et al. "Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice." Diabetes vol. 57,6 (2008): 1470-81. doi:10.2337/db07-1403
5. Cani, Patrice D et al. "Metabolic endotoxemia initiates obesity and insulin resistance." Diabetes vol. 56,7 (2007): 1761-72. doi:10.2337/db06-1491
6. Liu MJ, Yang JY, Yan ZH, et al. Recent findings in Akkermansia muciniphila-regulated metabolism and its role in intestinal diseases. Clin Nutr. 2022 Oct;41(10):2333-2344. doi: 10.1016/j.clnu.2022.08.029. Epub 2022 Sep 3. PMID: 36113229
7. Everard A, Cani PD. Gut microbiota and GLP-1. Rev Endocr Metab Disord. 2014 Sep;15(3):189-96. doi: 10.1007/s11154-014-9288-6. PMID: 24789701.

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Why is this important?

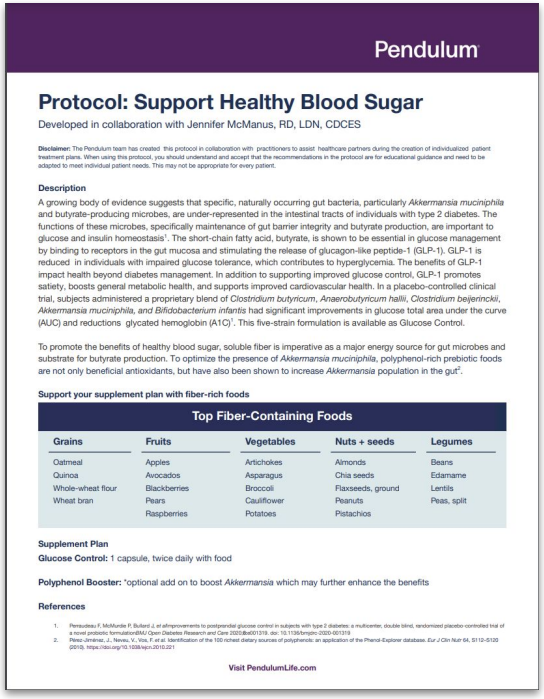
- **Metabolic dysfunction stems from gut permeability leading to chronic low-grade inflammation**
- A major protective factor against gut barrier permeability is the mucus layer
  - *Akkermansia muciniphila*
- Gut also mediates metabolic health through the production of certain metabolites, specifically butyrate
  - *Anaerobutyricum hallii, Clostridium butyricum, Clostridium beijerinckii*

Supplement Plan

- Pendulum Metabolic Daily: 1 capsule, once daily with food
- Pendulum Polyphenol Booster: optional add on to boost Akkermansia which may further enhance the benefits

*Support your supplement plan with high-fiber, polyphenol-rich foods

Support Healthy Blood Sugar



Pendulum

Protocol: Support Healthy Blood Sugar

Developed in collaboration with Jennifer McManus, RD, LDN, CDCES

Disclaimer: The Pendulum team has created this protocol in collaboration with practitioners to assist healthcare partners during the creation of individualized patient treatment plans. When using this protocol, you should understand and accept that the recommendations in the protocol are for educational guidance and need to be adapted to meet individual patient needs. This may not be appropriate for every patient.

Description

A growing body of evidence suggests that specific, naturally occurring gut bacteria, particularly *Akkermansia muciniphila* and butyrate-producing microbes, are under-represented in the intestinal tracts of individuals with type 2 diabetes. The functions of these microbes, specifically maintenance of gut barrier integrity and butyrate production, are important to glucose and insulin homeostasis[1]. The short-chain fatty acid, butyrate, is shown to be essential in glucose management by binding to receptors in the gut mucosa and stimulating the release of glucagon-like peptide-1 (GLP-1). GLP-1 is reduced in individuals with impaired glucose tolerance, which contributes to hyperglycemia. The benefits of GLP-1 impact health beyond diabetes management. In addition to supporting improved glucose control, GLP-1 promotes satiety, boosts general metabolic health, and supports improved cardiovascular health. In a placebo-controlled clinical trial, subjects administered a proprietary blend of *Clostridium butyricum, Anaerobutyricum hallii, Clostridium beijerinckii, Akkermansia muciniphila, and Bifidobacterium infantis* had significant improvements in glucose total area under the curve (AUC) and reductions glycated hemoglobin (A1C)[1]. This five-strain formulation is available as Glucose Control.

To promote the benefits of healthy blood sugar, soluble fiber is imperative as a major energy source for gut microbes and substrate for butyrate production. To optimize the presence of *Akkermansia muciniphila*, polyphenol-rich prebiotic foods are not only beneficial antioxidants, but have also been shown to increase *Akkermansia* population in the gut[2].

Support your supplement plan with fiber-rich foods

Top Fiber-Containing Foods

Grains	Fruits	Vegetables	Nuts + seeds	Legumes
Oatmeal	Apples	Artichokes	Almonds	Beans
Quinoa	Avocados	Asparagus	Chia seeds	Edamame
Whole-wheat flour	Blackberries	Broccoli	Flaxseeds, ground	Lentils
Wheat bran	Pears	Cauliflower	Peanuts	Peas, split
	Raspberries	Potatoes	Pistachios	

Supplement Plan

Glucose Control: 1 capsule, twice daily with food

Polyphenol Booster: *optional add on to boost *Akkermansia* which may further enhance the benefits

References

1. Perraudeau F, McMurdie P, Bullard J, *et al*improvements to postprandial glucose control in subjects with type 2 diabetes: a multicenter, double blind, randomized placebo-controlled trial of a novel probiotic formulationBMJ Open Diabetes Research and Care 2020;8:e001318. doi: 10.1136/bmjdrc-2020-001319
2. Pérez-Jiménez, J., Neveu, V., Vos, F. *et al.* Identification of the 100 richest dietary sources of polyphenols: an application of the Phenol-Explorer database. *Eur J Clin Nutr* 64, S112–S120 (2010). https://doi.org/10.1038/ejcn.2010.221

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Why is this important?

- Naturally occurring gut bacteria, particularly *Akkermansia muciniphila* and butyrate-producing microbes, are under-represented in individuals with diabetes
- Butyrate, is shown to be essential in glucose management by binding to receptors in the gut mucosa and stimulating the release of GLP-1
- **The functions of these microbes are important to glucose and insulin homeostasis**

Supplement Plan

- Glucose Control: 1 capsule, twice daily with food
- Polyphenol Booster: *optional add on to boost Akkermansia which may further enhance the benefits

*Support your supplement plan with high-fiber, polyphenol-rich foods

Thank you!

Q & A

Supporting Slides

Customer Case Study - Pendulum Glucose Control

Diagnosis: LADA (LADA Latent AutoImmune Diabetes in Adults)
Medications: Januvia, Verapamil, atorvastatin 20mg, acarbose, zyrtec, singular, azsteline, singular, proventil
Lifestyle: Busy working mother, not a lot of time to prioritize changes to her diet

Participated in CGM Education program beta

CGM Results (12/22/21 - 3/19/22)
GMI (Glucose Management Indicator): 6.6% → 5.9%
TIR (Time in Range) : 90.3% →98.6%
Average Glucose: 139 → 109
Glucose Variability: 21.6% →20.6%

Customer Case Study - Akkermansia

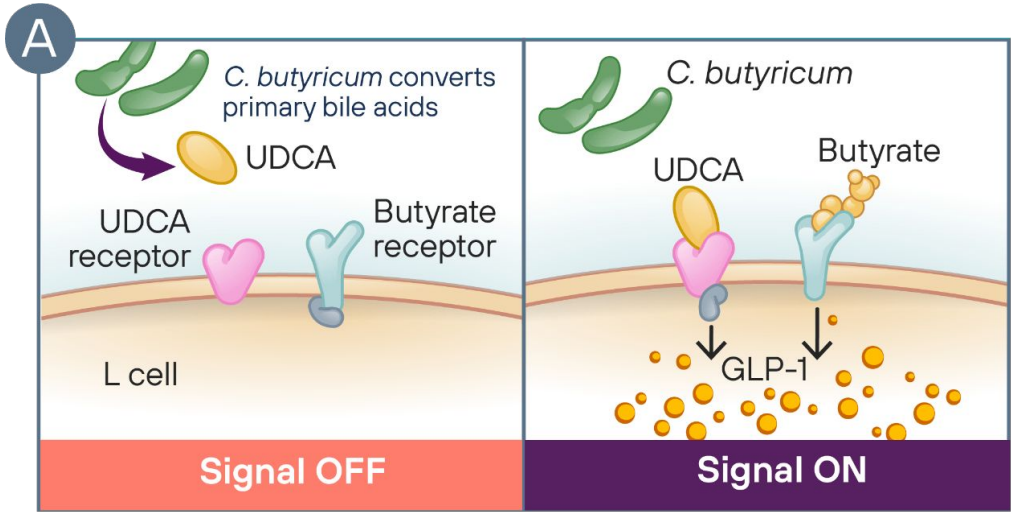
- **Case abstract** - Customer is a 32 YO F who started taking Akkermansia for a variety of reasons and has an extensive health history. She was dx with hypothyroidism 12 years ago (2010) after living in Indonesia (2009) and returning to the US with severe headaches. She was found to have 4 parasites (3 in her intestines and 1 in her brain). After having brain surgery to remove the parasitic infection, she developed adrenal system issues (i.e. hypothyroidism). She also has a hx of extensive skin infections (including staph), and she has been on-off Doxycycline for many years. She has tried many different skincare routines, including seeing an esthetician.
- **Case history/Eating behavior & lifestyle information** - **Eating behavior & lifestyle information** - Customer follows a gluten-free, dairy-free, egg-free, and soy-free diet with varied/balanced meals three times per day. Her breakfast consists of gluten-free oatmeal and her lunches and dinners usually consist of a lean protein, a starch, and a vegetable. The customer eats most meals at home and tries to cook as much as she can due to sodium intake sensitivity and water retention. The customer does not consume much added sugar (drinks black coffee and unsweetened tea, limits alcohol, and no desserts, aside from dark chocolate). She also does not snack much, and if doing so, chooses to have hummus with vegetables and pita bread or charcuterie and cheese. She drinks plenty of fluids each day as well (100-120 fluid oz.). She is considered low-active and enjoys pilates and going to the gym.
- **Assessment of nutrition status** - Her diet could increase in dietary fiber. Customer dislikes fruit, and she was practicing an elimination diet of sorts for awhile, which could lead to vitamin and mineral deficiencies.
- **Results/Outcomes -** After 1.5 months of taking Akkermansia, the customer has reported a wt. loss of 12 lbs., increased energy, skin improvements, reduced bloating, and formed stools.

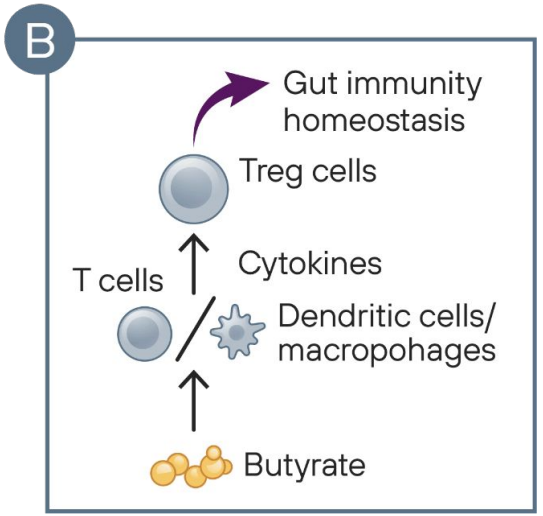
Study Design: Product Formulation

Ingredients	Placebo	WBF-010	WBF-011
Colloidal silicon dioxide	●	●	●
Inulin Prebiotic		●	●
Clostridium beijerinckii (CBEI) Butyrate producing		●	●
Clostridium butyricum (CBUT) Butyrate producing		●	●
Bifidobacterium infantis (BINF) Support strain		●	●
Akkermansia muciniphila (AMUC) Mucin regulator			●
Anaerobutyricum hallii (AHAL) Butyrate producing			●

Study Design: Demographics

Intent-to-treat	Placebo (n = 26)	3-strains (n = 27)	5-strains (n = 23)
Age (yr)	53.7±1.5	49.3±2.3	51.3±1.7
Female (no. (%))	15 (57.7)	18 (66.7)	13 (56.5)
Weight (kg)	93.4±4.5	92.7±4.1	87.6±4.5
Body-mass index (kg/m^2)	33.4±1.1	34.4±1.1	31.9±1.0
Glycated Hemoglobin (%)	8.9 ± 0.3	8.5± 0.3	8.8±0.3
Fasting glucose (mg/dL)	208.8±12.8	179.6±12.5	179. ±10.9
# D.E./+Met/+SFU	9/10/7	1/20/6	4/16/3


A
C. butyricum converts primary bile acids
UDCA
UDCA receptor
Butyrate receptor
L cell
Signal OFF
C. butyricum
UDCA
Butyrate
GLP-1
Signal ON


B
Gut immunity homeostasis
Treg cells
Cytokines
T cells
Dendritic cells/ macropohages
Butyrate

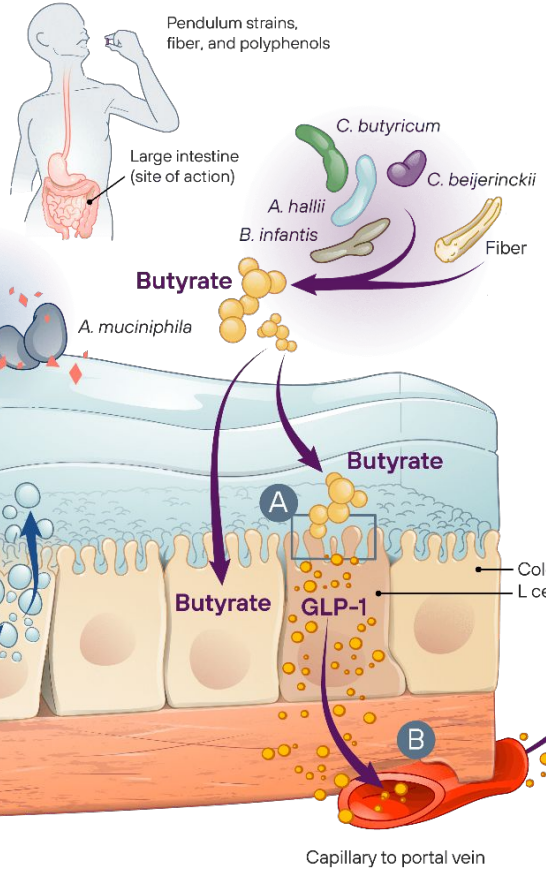
Overview of gut microbiome function

Pendulum Strains

Mucin fortifier
Akkermansia muciniphila

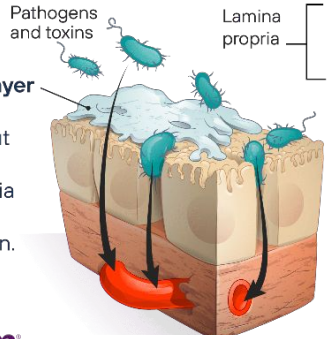
Butyrate producers
Clostridium butyricum
Clostridium beijerinckii
Anaerobutyricum hallii

Primary fermenter
Bifidobacterium infantis

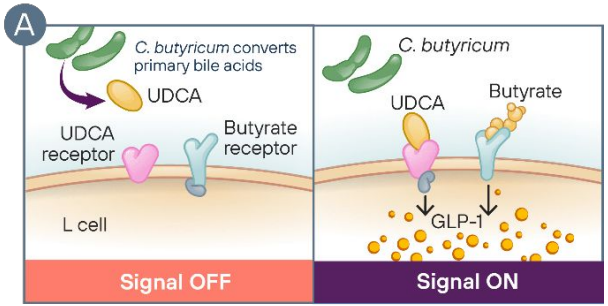


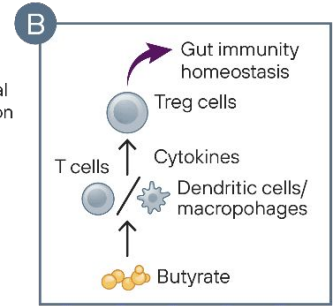
↑ Mucin production

↑ Barrier function
↑ Gut immunity homeostasis



Thinning mucin layer can lead to the development of gut dysbiosis, allowing pathogenic bacteria to infiltrate the systemic circulation.



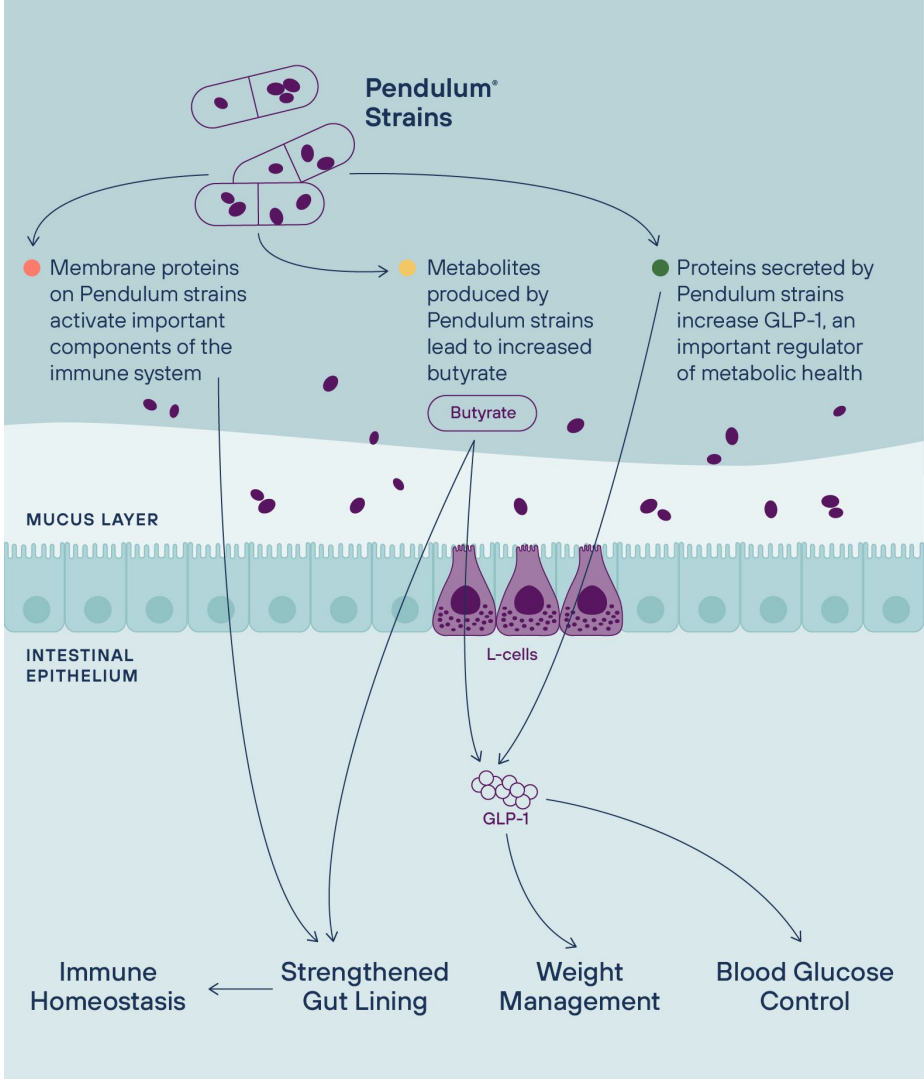


↑ GLP-1

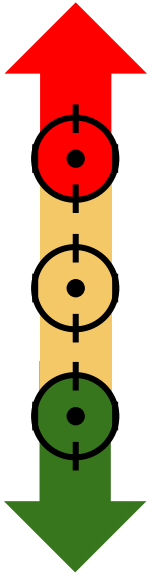
↑ Insulin secretion
↑ Insulin sensitivity
↓ Glucagon secretion
↑ β-cell health
↑ Satiety
↑ Improved glucose control
↑ Improved metabolic health
↓ Metabolic endotoxemia
↓ Systemic inflammation

Pendulum's Proprietary Strains Restore Key Functions

- **Increase synthesis of short chain fatty acids (SCFAs) such as butyrate**
  - *Clostridium butyricum*
  - *Clostridium beijerinckii*
  - *Anaerobutyricum hallii*
- **Mucin fortifier**
  - *Akkermansia muciniphila*
- **Primary Fermenter**
  - *Bifidobacterium infantis*



Pendulum Glucose Control: Clinically Proven



A1c Test Results

Diabetes
6.5% or higher

Prediabetes
5.7 to 6.4%

Normal
Below 5.7%

	Pendulum Glucose Control	Metformin	SGLT2	GLP1	Insulin
A1C reduction	-0.6%	-0.7-1.2%	0.6-0.9%	-0.7-1.6%	-0.7-1.0%
AUC reduction	-33%	-20%	-23%	-50%	-32%
Product	Medical Probiotic	Drug	Drug	Drug (injection)	Drug (injection)
Side Effects	None known	GI distress Lactic Acidosis	Diabetic ketoacidosis Dehydration Genital infections	Pancreatitis Injection site reaction Nausea	Hypoglycemia Lipoatrophy ↑ CV risk Weight gain

Pendulum Glucose Control has efficacy for people with diabetes