Harnessing GLP-1 in the digestive tract for better metabolic health

Orville Kolterman, MD
October 2023

Introduction

Metabolic health involves optimal functioning of the complex processes by which the body processes food and uses energy. Maintenance of healthy metabolism is critically important for all individuals, not only for helping avoid (or manage) metabolic diseases but also for maintaining well-being and vitality.

Among the many recognized hormones contributing to metabolic health, glucagon-like peptide 1 (GLP-1) is a well-studied hormone produced in the human gut, with several critical functions in human metabolic health. One of its most recognized clinical effects is to enhance satiety, leading to decreased energy intake (Shah and Vella, 2014). GLP-1 function is altered in type 2 diabetes, obesity, and other conditions, and has long been of interest for therapeutic use. However, for many years its use as a drug was hindered because it is rapidly degraded in the body. Once drugs were developed that overcame the challenge of rapid degradation, GLP-1 was successfully leveraged to improve metabolic health and induce weight loss (Drucker et al. 2017). However, the drug therapy induces circulation of the hormone/agonists at much higher concentrations than would occur naturally in the body, and side effects are troublesome to many patients. Recently, research has shown how GLP-1 interacts in the complex environment of the gut, including with the gut microbial ecosystem (Abdalqadir and Adeli, 2022). In the gut, specific bacterial taxa, notably *Akkermansia* and butyrate producing strains such as *Clostridium butyricum*, may offer a physiological way to stimulate GLP-1 production to mimic more closely its normal concentration and functions in the human body resulting in more natural regulation of metabolic health.

GLP-1 Overview

GLP-1 is a 30 amino acid peptide hormone that is produced in response to nutrient ingestion, through cleavage of the prohormone proglucagon within the enteroendocrine L cells located within the epithelial lining of the intestine – predominantly in the colon and terminal ileum. It is a member of the incretin hormone family known to augment β-cell glucose-dependent insulin secretion in response to nutrient ingestion. Since this stimulation of insulin secretion is glucose-dependent, insulin levels decrease in parallel with the decline in the prevailing plasma glucose concentration. This feature blunts the likelihood of inducing hypoglycemia and its attendant risks, an attribute that has significant clinical benefit (Holst et al. 2021).

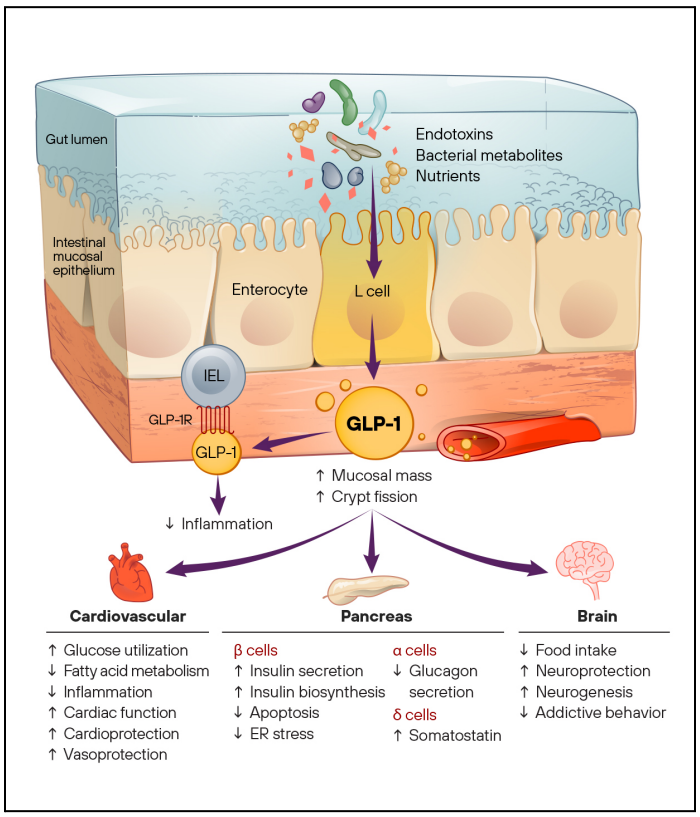
While researchers initially demonstrated that GLP-1 stimulated glucose-dependent insulin secretion following nutrient ingestion, GLP-1 has proved to be a multifaceted hormone with effects throughout the body, impacting metabolic control and numerous other functions (Drucker and Holst, 2023). In addition to stimulating postprandial insulin secretion, GLP-1 suppresses the secretion of glucagon, the pancreatic hormone that opposes the effects of insulin to guard against hypoglycemia by increasing the release of glucose by the liver. GLP-1 also reduces the rate at which nutrients pass through the stomach (gastric emptying), thus restraining the absorption rate of ingested nutrients into the peripheral circulation (Drucker et al. 2017).

Importantly, GLP-1 also exerts effects within the central nervous system to promote satiety (Richards et al. 2014). These appetite modulating effects are mediated in part by afferent signaling via the vagus nerve combined with the effects of GLP-1 entering through fenestrations in the blood brain barrier near the hindbrain region of the *area postrema* where interactions with neuronal GLP-1 receptors activate

well established pathways involved in appetite control.

As shown in Figure 1, GLP-1 has multiple other beneficial effects.

Figure 1. Production and functions of glucagon-like peptide 1 (GLP-1) inside the digestive tract. The hormone is produced by the L cells in the epithelial layer and exert multiple effects throughout the body. (IEL: intraepithelial lymphocytes; GLP-1R: glucagon-like peptide-1 receptor.)



Metabolic Effects of GLP-1

The observation in the mid to late 1990s that GLP-1 lowered both fasting and postprandial glucose concentrations in both rodents and humans (Nauck et al. 1993, Nauck et al. 1998) led to immediate interest in harnessing this phenomenon to assist in the management of hyperglycemia in patients with T2D (Drucker et al. 2017). This interest intensified with the subsequent demonstrations that the hormone also decreased glucagon following meals, in addition to restraining gastric emptying and increasing satiety. And, in contrast to the hypoglycemic risk that accompanies exogenous insulin and sulfonylurea therapy, GLP-1 achieved these benefits without increasing the risk for hypoglycemia since the stimulation of insulin secretion is glucose-dependent with the stimulatory effect dissipating in parallel with the subsequent fall of plasma glucose concentrations towards normal. This attribute of GLP-1 allows the fall of plasma glucose concentrations to plateau within the normal range without the occurrence of clinically meaningful hypoglycemia. The safety margin is further enhanced by the suppression of glucagon secretion and restraint of gastric emptying also receding as plasma glucose concentrations enter the normal range, allowing endogenous glucagon secretion and orally administered glucose to reverse hypoglycemia should it occur (Degn et al. 2004).

GLP-1 for the Management of Type 2 Diabetes

The combination of glucose-dependent stimulation of insulin secretion coupled with suppression of glucagon and restraint of the rate of nutrient absorption provide excellent control of the rise in plasma glucose concentrations following meals (Drucker, 2018). These attributes of GLP-1, as well as the ability to lower fasting glucose concentrations, coupled with a low risk of hypoglycemia, were first demonstrated through intravenous infusion of the peptide, showing the potential of a GLP-1 based therapy for the management of T2D (Nauck et al. 1993, Nauck et al. 1998).

However, native GLP-1 has a circulating half-life that is measured in minutes due to its susceptibility to enzymatic degradation by dipeptidyl peptidase IV (DPP-IV) which is ubiquitous throughout the body. Thus, molecular entities with longer half-lives which retained the conformational requirements for binding to the GLP-1 receptor were necessary. This was achieved through two routes. First, exendin 4, a naturally occurring peptide in the salivary secretions of the gila monster lizard (*Heloderma Suspectum*), was found to be naturally resistant to degradation by DPP-IV with a circulating half-life that delivers therapeutic efficacy with twice daily injections. Synthetic exendin 4, known as exenatide, was developed and received FDA approval as the first

GLP-1 receptor agonist in 2005 (McBrayer & Tal-Gan, 2017). The second approach consists of conjugating a 16-carbon fatty acid moiety to native GLP-1 to foster non-covalent binding to circulating plasma proteins, primarily albumin, creating a "slow-release depot" with a pharmacokinetic profile that supports once-daily administration by injection (Drucker et al. 2017). Liraglutide was the first approved drug employing this approach, with several additional products following shortly thereafter that utilized a similar approach. Further pharmaceutical development leveraging newer approaches to injectable drug delivery produced products that provided the desired efficacy with once-weekly administration. More recently, an oral formulation of semaglutide has entered the market, but its uptake has been restrained by the requirement for more complicated dosing algorithms.

GLP-1 agonism is cardioprotective as demonstrated by a reduction in major adverse cardiovascular events (MACE) in patients with type 2 diabetes (T2D) during long-term exposure to various GLP-1 receptor agonists and for treatment of type 2 diabetes (T2D) (Kristensen et al. 2019).

Lastly, the therapeutic attractiveness of GLP-1 receptor agonists is further augmented by the limited risk for hypoglycemia and the likelihood of weight loss instead of weight gain (McBrayer and Tal-Gan, 2017).

GLP-1 for the Management of Obesity

As noted above, GLP-1 analogues stand apart from most other antidiabetic agents in that they do not drive weight gain. In fact, the therapeutic use of GLP-1 agents is usually associated with weight loss. This observation led to interest in the potential for these agents to be used in weight control programs. This led to exploration of higher doses of liraglutide and semaglutide for this purpose, with impressive results (>15% reduction in body weight in subjects with BMI>30 kg/m^2) with subsequent approval of those formulations for use in the management of obesity (Moore et al. 2023). Tirzepatide, which combines a GLP-1 and GIP (glucose dependent insulinotropic polypeptide) agonist in a single molecule has demonstrated even more robust weight loss (>20% reduction from baseline weight in obese subjects without diabetes) (Jastreboff et al. 2022). This compound has been granted Fast Track Designation by FDA for use in obesity with anticipated approval by the end of 2023.

All approved GLP-1 agonists have so far demonstrated a good safety profile even though they produce circulating levels of GLP-1 agonism that are about ten times greater than the physiological GLP-1 concentrations seen in healthy subjects. Side effects with these agents are primarily related to gastrointestinal tolerability, with nausea and/or vomiting occurring in over 25% of subjects in most studies (Tran et al. 2017). Most afflicted individuals find that these side effects dissipate or cease during the initial month of therapy. After considering the benefits and risks of these agents, only ~5% of patients elect to discontinue therapy. However, some healthcare professionals are concerned about the growing popularity of these drugs, since the mechanisms by which they achieve substantial weight loss are not completely understood. The long-term effects of high circulating levels of GLP-1 are still unknown, and additional adverse reactions (such as intestinal blockages) have been very recently identified. Moreover, the American Society of Anesthesiologists has stated concerns about case reports that GLP-1 agonists can increase the risk of regurgitation and pulmonary aspiration of stomach contents during anesthesia. Given these potential risks, non-drug approaches to GLP-1 stimulation are worth investigating.

Gut Microbiome Contributions to GLP-1

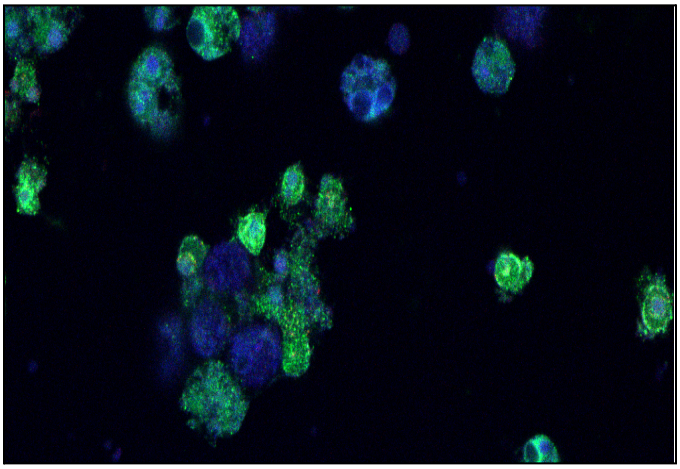
Cross-sectional population studies profiling the gut microbiome have repeatedly demonstrated decreased diversity in individuals exhibiting obesity and/or type 2 diabetes (Huda et al. 2021). When fecal transplants from these subjects to germ free mice are performed, a similar microbiome is established in the rodent recipients, along with features of the human donor's phenotype, i.e., development of obesity (Ridaura et al. 2013) and/or diabetes. Salient characteristics of these microbiomes include a marked decrease or absence of *Akkermansia* as well as butyrate-producing

strains, compared to healthy subjects. Subsequent reversal of those changes in the rodent models' microbiomes attenuate or reverse the metabolic abnormalities. Similar short-term improvements in metabolic parameters have been observed in human subjects with T2D following both single and multiple fecal transplants (Hanssen et al. 2021).

The fecal transplant data demonstrates that constituents of fecal material from healthy subjects contain elements capable of improving metabolic control. Characterization of the gut microbiome profiles of obese subjects with or without T2D have repeatedly shown decreased diversity with significant decreases in both *Akkermansia* and strains known to produce short chain fatty acids (SCFAs) by the fermentation of dietary fiber. *Akkermansia* as well as bacterial producers of the SCFA butyrate such as *C. butyricum* have been shown to stimulate GLP-1 secretion by the intestinal L cells (Stoeva et al. 2021, Yoon et al. 2021). *Akkermansia* stimulates GLP-1 secretion by these enteroendocrine cells via three distinct mechanisms: the release of a stimulatory peptide referred to as P9, a component of its outer wall identified as Amuc_1100, and the release of extracellular vesicles (Keshavarz Azizi Raftar et al. 2021). Interestingly, one study showed that *Akkermansia* that has been pasteurized prior to administration retains the ability to stimulate GLP-1 secretion (Depommier et al. 2019). However, the administration of killed bacteria limits the stimulatory capacity to those present in the administered product whereas in theory, administration of live bacteria allows for amplification of the stimulus via continuous production within the gut. Multiple other studies have demonstrated the ability of butyrate producing gut microbes to also increase GLP-1 secretion by L cells in rodent models (Yadav et al. 2013).

These observations raised the possibility that a targeted probiotic containing *Akkermansia* along with butyrate-producing specialist strains such as *C. butyricum* could assist in improving metabolic control. A recently published human study confirmed this by demonstrating that administration of a five-strain probiotic containing *Akkermansia* and three butyrate-producing strains for twelve weeks led to significant improvement in metabolic control (reduction in plasma glucose concentrations following ingestion of a mixed meal accompanied by reduction in A1c, the gold standard for long-term glucose control) in subjects with type 2 diabetes treated with metformin (Perraudeau et al. 2020). These improvements in metabolic control were accompanied by increased fecal and plasma butyrate concentrations (McMurdie et al. 2022). Investigators also saw an increase in plasma levels of the secondary bile acid ursodeoxycholate, which has also shown the ability to stimulate GLP-1 secretion from L cells (Murakami et al. 2013).

It has recently been shown directly that Pendulum's *Akkermansia* stimulates GLP-1 production in cultured human L cells (internal data). Dose dependent GLP-1 secretion was observed in an established human L cell line when exposed to media supernatant in which *Akkermansia* had been grown. Representative stained L cells with and without *Akkermansia* supernatant are shown in Figure 2.



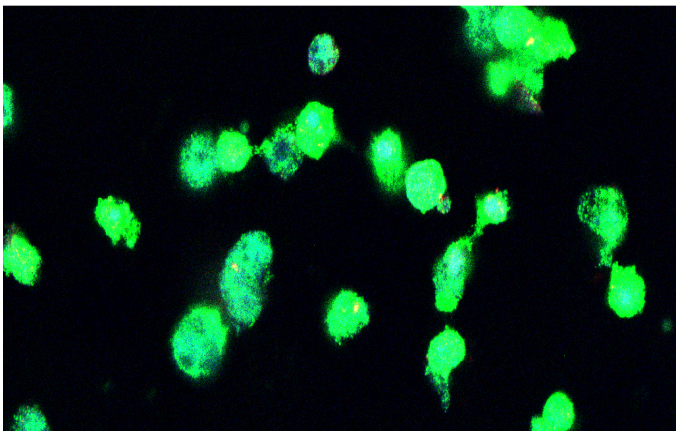


Figure 2. Cultured L cells incubated with control growth media not exposed to *Akkermansi*a (A) and supernatant growth media from *Akkermansia* culture (B). The cells were fixed and labeled with anti-GLP-1 polyclonal antibody conjugated with alexa 488 fluorophore.

Together, these data suggest a natural way to harness GLP-1 in the digestive tract: using targeted probiotic formulations in the gut to naturally stimulate GLP-1 production. Such an approach would avoid the high circulating levels of GLP-1 induced by current drug therapies and likely reduce side effects, mimicking more closely GLP-1's normal concentration and functions in the human body.

Summary

GLP-1 is shown to play an important role in maintaining metabolic homeostasis. Its therapeutic utility was only realized after the development of approaches to prevent its rapid degradation by the ubiquitous DPP-IV enzyme. Once that challenge was met, the expectations for leveraging its activity to improve glucose control and induce weight loss were realized. Increasing knowledge of the gut microbiome, however, has revealed that *Akkermansia* and microbial strains that produce butyrate (for example, *C. butyricum*) can augment GLP-1 production through natural stimulation of the L cells. This path to increased GLP-1 production introduces the hormone into the body via its normal pathway, allowing its effects as a hormone, its communications with the intestinal immune system and its interactions with both the peripheral and central nervous systems to be exerted via the natural physiological processes. This approach may be an effective alternative solution in allowing moderate benefits of GLP-1 to be realized without the need for the exaggerated circulating concentration required to achieve the efficacy of the synthetic analogs – and perhaps avoiding the negative side effects of these GLP-1 drugs. Given the complexity of maintaining metabolic homeostasis in the body and the important contributions of GLP-1 to overall metabolic health, efforts to approximate the hormone's natural functioning in the human gut are highly worthwhile.

Orville Kolterman, MD is the Chief Medical Officer at Pendulum Therapeutics and Adjunct Clinical Professor of Medicine at the University of California, San Diego. Dr. Kolterman is a past President of the California Affiliate of the ADA and currently serves as member of the Board of Directors of GlySens and the diaTribe Foundation. Dr. Kolterman received his M.D. from Stanford University and also completed his fellowship in Endocrinology and Metabolism at Stanford Hospital.

References

Abdalqadir N, Adeli K. GLP-1 and GLP-2 Orchestrate Intestine Integrity, Gut Microbiota, and Immune System Crosstalk. Microorganisms. 2022 Oct 19;10(10):2061. doi: 10.3390/microorganisms10102061. PMID: 36296337; PMCID: PMC9610230.

Degn KB, Brock B, Juhl CB, Djurhuus CB, Grubert J, Kim D, Han J, Taylor K, Fineman M, Schmitz O. Effect of intravenous infusion of exenatide (synthetic exendin-4) on glucose-dependent insulin secretion and counterregulation during hypoglycemia. Diabetes. 2004 Sep;53(9):2397-403. doi: 10.2337/diabetes.53.9.2397. PMID: 15331551.

Depommier C, Everard A, Druart C, Plovier H, Van Hul M, Vieira-Silva S, Falony G, Raes J, Maiter D, Delzenne NM, de Barsy M, Loumaye A, Hermans MP, Thissen JP, de Vos WM, Cani PD. Supplementation with Akkermansia muciniphila in overweight and obese human volunteers: a proof-of-concept exploratory study. Nat Med. 2019 Jul;25(7):1096-1103. doi: 10.1038/s41591-019-0495-2. Epub 2019 Jul 1. PMID: 31263284; PMCID: PMC6699990.

Drucker DJ. Mechanisms of Action and Therapeutic Application of Glucagon-like Peptide-1. Cell Metab. 2018 Apr 3;27(4):740-756. doi: 10.1016/j.cmet.2018.03.001. PMID: 29617641.

Drucker DJ, Habener JF, Holst JJ. Discovery, characterization, and clinical development of the glucagon-like peptides. J Clin Invest. 2017 Dec 1;127(12):4217-4227. doi: 10.1172/JCI97233. Epub 2017 Dec 1. PMID: 29202475; PMCID: PMC5707151.

Drucker DJ, Holst JJ. The expanding incretin universe: from basic biology to clinical translation. Diabetologia. 2023 Oct;66(10):1765-1779. doi: 10.1007/s00125-023-05906-7. Epub 2023 Mar 28. PMID: 36976349.

Hanssen NMJ, de Vos WM, Nieuwdorp M. Fecal microbiota transplantation in human metabolic diseases: From a murky past to a bright future? Cell Metab. 2021 Jun 1;33(6):1098-1110. doi: 10.1016/j.cmet.2021.05.005. PMID: 34077717.

Holst JJ, Gasbjerg LS, Rosenkilde MM. The Role of Incretins on Insulin Function and Glucose Homeostasis. Endocrinology. 2021 Jul 1;162(7):bqab065. doi: 10.1210/endocr/bqab065. PMID: 33782700; PMCID: PMC8168943.

Huda MN, Kim M, Bennett BJ. Modulating the Microbiota as a Therapeutic Intervention for Type 2 Diabetes. Front Endocrinol (Lausanne). 2021 Apr 7;12:632335. doi: 10.3389/fendo.2021.632335. PMID: 33897618; PMCID: PMC8060771.

Jastreboff AM, Aronne LJ, Ahmad NN, Wharton S, Connery L, Alves B, Kiyosue A, Zhang S, Liu B, Bunck MC, Stefanski A; SURMOUNT-1 Investigators. Tirzepatide Once Weekly for the Treatment of Obesity. N Engl J Med. 2022 Jul 21;387(3):205-216. doi: 10.1056/NEJMoa2206038. Epub 2022 Jun 4. PMID: 35658024.

Keshavarz Azizi Raftar S, Ashrafian F, Yadegar A, Lari A, Moradi HR, Shahriary A, Azimirad M, Alavifard H, Mohsenifar Z, Davari M, Vaziri F, Moshiri A, Siadat SD, Zali MR. The Protective Effects of Live and Pasteurized Akkermansia muciniphila and Its Extracellular Vesicles against HFD/CCl4-Induced Liver Injury. Microbiol Spectr. 2021 Oct 31;9(2):e0048421. doi: 10.1128/Spectrum.00484-21. Epub 2021 Sep 22. PMID: 34549998; PMCID: PMC8557882.

Kristensen SL, Rørth R, Jhund PS, Docherty KF, Sattar N, Preiss D, Køber L, Petrie MC, McMurray JJV. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. Lancet Diabetes Endocrinol. 2019 Oct;7(10):776-785. doi: 10.1016/S2213-8587(19)30249-9. Epub 2019 Aug 14. Erratum in: Lancet Diabetes Endocrinol. 2020 Mar;8(3):e2. PMID: 31422062.

McBrayer DN, Tal-Gan Y. Recent Advances in GLP-1 Receptor Agonists for Use in Diabetes Mellitus. Drug Dev Res. 2017 Sep;78(6):292-299.

doi: 10.1002/ddr.21404. Epub 2017 Aug 8. PMID: 28786125; PMCID: PMC5602594.

McMurdie PJ, Stoeva MK, Justice N, Nemchek M, Sieber CMK, Tyagi S, Gines J, Skennerton CT, Souza M, Kolterman O, Eid J. Increased circulating butyrate and ursodeoxycholate during probiotic intervention in humans with type 2 diabetes. BMC Microbiol. 2022 Jan 8;22(1):19. doi: 10.1186/s12866-021-02415-8. PMID: 34996347; PMCID: PMC8742391.

Moore PW, Malone K, VanValkenburg D, Rando LL, Williams BC, Matejowsky HG, Ahmadzadeh S, Shekoohi S, Cornett EM, Kaye AD. GLP-1 Agonists for Weight Loss: Pharmacology and Clinical Implications. Adv Ther. 2023 Mar;40(3):723-742. doi: 10.1007/s12325-022-02394-w. Epub 2022 Dec 24. PMID: 36566341.

Murakami M, Une N, Nishizawa M, Suzuki S, Ito H, Horiuchi T. Incretin secretion stimulated by ursodeoxycholic acid in healthy subjects. Springerplus. 2013 Dec;2(1):20. doi: 10.1186/2193-1801-2-20. Epub 2013 Jan 22. PMID: 23450079; PMCID: PMC3579475.

Nauck MA, Kleine N, Orskov C, Holst JJ, Willms B, Creutzfeldt W. Normalization of fasting hyperglycaemia by exogenous glucagon-like peptide 1 (7-36 amide) in type 2 (non-insulin-dependent) diabetic patients. Diabetologia. 1993 Aug;36(8):741-4. doi: 10.1007/BF00401145. PMID: 8405741.

Nauck MA, Sauerwald A, Ritzel R, Holst JJ, Schmiegel W. Influence of glucagon-like peptide 1 on fasting glycemia in type 2 diabetic patients treated with insulin after sulfonylurea secondary failure. Diabetes Care. 1998 Nov;21(11):1925-31. doi: 10.2337/diacare.21.11.1925. PMID: 9802745.

Perraudeau F, McMurdie P, Bullard J, Cheng A, Cutcliffe C, Deo A, Eid J, Gines J, Iyer M, Justice N, Loo WT, Nemchek M, Schicklberger M, Souza M, Stoneburner B, Tyagi S, Kolterman O. 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 Res Care. 2020 Jul;8(1):e001319. doi: 10.1136/bmjdrc-2020-001319. PMID: 32675291; PMCID: PMC7368581.

Richards P, Parker HE, Adriaenssens AE, Hodgson JM, Cork SC, Trapp S, Gribble FM, Reimann F. Identification and characterization of GLP-1 receptor-expressing cells using a new transgenic mouse model. Diabetes. 2014 Apr;63(4):1224-33. doi: 10.2337/db13-1440. Epub 2013 Dec 2. PMID: 24296712; PMCID: PMC4092212.

Ridaura VK, Faith JJ, Rey FE, Cheng J, Duncan AE, Kau AL, Griffin NW, Lombard V, Henrissat B, Bain JR, Muehlbauer MJ, Ilkayeva O, Semenkovich CF, Funai K, Hayashi DK, Lyle BJ, Martini MC, Ursell LK, Clemente JC, Van Treuren W, Walters WA, Knight R, Newgard CB, Heath AC, Gordon JI. Gut microbiota from twins discordant for obesity modulate metabolism in mice. Science. 2013 Sep 6;341(6150):1241214. doi: 10.1126/science.1241214. PMID: 24009397; PMCID: PMC3829625.

Shah M, Vella A. Effects of GLP-1 on appetite and weight. Rev Endocr Metab Disord. 2014 Sep;15(3):181-7. doi: 10.1007/s11154-014-9289-5. PMID: 24811133; PMCID: PMC4119845.

Stoeva MK, Garcia-So J, Justice N, Myers J, Tyagi S, Nemchek M, McMurdie PJ, Kolterman O, Eid J. Butyrate-producing human gut symbiont, Clostridium butyricum, and its role in health and disease. Gut Microbes. 2021 Jan-Dec;13(1):1-28. doi: 10.1080/19490976.2021.1907272. PMID: 33874858; PMCID: PMC8078720.

Tran KL, Park YI, Pandya S, Muliyil NJ, Jensen BD, Huynh K, Nguyen QT. Overview of Glucagon-Like Peptide-1 Receptor Agonists for the Treatment of Patients with Type 2 Diabetes. Am Health Drug Benefits. 2017 Jun;10(4):178-188. PMID: 28794822; PMCID: PMC5536194.

Yadav H, Lee JH, Lloyd J, Walter P, Rane SG. Beneficial metabolic effects of a probiotic via butyrate-induced GLP-1 hormone secretion. J Biol Chem. 2013 Aug 30;288(35):25088-25097. doi:

10.1074/jbc.M113.452516. Epub 2013 Jul 8. PMID: 23836895; PMCID: PMC3757173.

Yoon HS, Cho CH, Yun MS, Jang SJ, You HJ, Kim JH, Han D, Cha KH, Moon SH, Lee K, Kim YJ, Lee SJ, Nam TW, Ko G. Akkermansia muciniphila secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice. Nat Microbiol. 2021 May;6(5):563-573. doi: 10.1038/s41564-021-00880-5. Epub 2021 Apr 5. PMID: 33820962.