MICROBIOLOGY

Microbial dipeptidyl peptidases of the S9B family as host-microbe isozymes

Mashael R. Aljumaah[1,2,3], Jeffery Roach[1], Yunan Hu[1], John Gunstad[4], M. Andrea Azcarate-Peril[1]*

Human dipeptidyl peptidase 4 (hDPP-4) has been a pharmacological target for metabolic diseases, particularly diabetes, since the early 2000s. As a ubiquitous enzyme found in both prokaryotic and eukaryotic organisms, hDPP-4 plays crucial roles in host homeostasis and disease progression. While many studies have explored hDPP-4's properties, research on gut microbially derived DPP-4 (mDPP-4) remains limited. This review discusses the significance of mDPP-4 and its health implications, analyzing crystal structures of mDPP-4 in comparison to human counterparts. We examine how hDPP-4 inhibitors could influence gut microbiome composition and mDPP-4 activity. Additionally, this review connects ongoing discussions regarding DPP-4 substrate specificity and potential access routes for mDPP-4, emphasizing the urgent need for further research on mDPP-4's role in health and improve the precision of DPP-4 inhibitor therapies.

INTRODUCTION

Gut microbes provide complementary functionality and supply enzymes and metabolites, which may be lacking in the host, to ensure gut homeostasis and systemic well-being. The idea that the host and microbes share enzymatic capabilities in this symbiotic relationship is not new. We have shown that lactose-sensitive individuals who have reduced expression of intestinal lactase can consume prebiotics that enhance functionally similar β-galactosidase–carrying beneficial bacteria such as *Bifidobacterium* sp. As a result, they can digest lactose without experiencing adverse side effects (*1*, *2*). A more structured notion of host-microbe isozymes (HMIs) arose recently when Wang *et al.* (*3*) set up a pipeline to detect functional similarities between bacterial and human activities and identified homologous protein of human dipeptidyl peptidase 4 (hDPP-4) with different amino acid sequences in bacteria and fungi. HMI can be defined broadly as enzymes with functions similar to those of the host; however, DNA sequence–based identification of isozymes may not be reliable due to the lack of sequence conservation in enzymes with similar functionality.

Dipeptidyl peptidase 4 (DPP-4) is a proline-specific serine protease that selectively cleaves dipeptides from peptides and proteins containing proline or alanine in the N terminus next to last (P1) position, resulting in the altered functional activity of the cleaved substrates and further degradation of macromolecules by other peptidases. hDPP-4 is a multifunctional enzyme proposed as a substantial modulator of metabolic and systematic functions and, potentially, of the gut-brain axis (*4*). hDPP-4 has been shown to modulate the functionality of more than 40 bioactive substrates, many of which are involved in gut homeostasis, glucose metabolism, cognition, and behavior (*5*, *6*).

Although the general process involved in protein digestion and the most crucial host proteases have been well studied, the diversity and functionality of microbial proteolytic enzymes in the gut and their role in health and disease remain undefined. Protein digestion in the human gastrointestinal tract starts in the stomach. It involves breaking down complex protein molecules into peptides consisting of several amino acids and then into individual amino acids. The stomach secretes pepsins, responsible for ~10 to 15% of protein digestion, and is most active during the first hour of digestion. Their ability to break down protein is limited to an acidic environment with a pH between 1.8 and 3.5. The pancreas secretes trypsins, which are more potent than pepsins, and, as a result, most of the protein digestion occurs in the duodenum and upper jejunum. Inactive protease precursors are generated by the pancreas, which become active by interacting with enterokinases, enzymes secreted by the microvillous component of the enterocytes. Trypsinogen is activated by enterokinase in the intestine and then activates other pancreatic proteases like chymotrypsin and elastase. These endopeptidases break down protein chains into peptides. Carboxypeptidases, secreted by the pancreas, break down the peptides into smaller molecules and individual amino acids. Ultimately, this process reduces dietary proteins to small polypeptide chains of two to six amino acids and single amino acids. Dipeptides and tripeptides are transported into enterocytes by carrier proteins that require energy. Small peptides with few amino acids are directly absorbed, while the more substantial part of peptide breakdown occurs within the enterocyte. Small peptides are absorbed faster than amino acids; some amino acids have specific transport systems while others share one.

The most accepted classification of peptidases is collected in the MEROPS database (Release 12.5) (*7*), a manually curated information resource for proteolytic enzymes, their inhibitors, and substrates. The classification scheme clusters homologous peptidase and protein inhibitor sequences into peptidase and inhibitor "species." The species with unique identifiers are then clustered into families, grouped into clans. A family contains related sequences based on common ancestry, and a clan contains related tertiary structures based on their catalytic mechanisms.

Peptidases are categorized into six types on the basis of the nature of their catalytic site (aspartic, cysteine-type, metal, serine-type, threonine-type, and unassigned peptidases) (*8*). Within serine-type peptidases, we focused on peptidases that remove X-Pro dipeptides from the N terminus of peptides containing proline or alanine at the penultimate position, which are classified into S9B and S15 families according to structural and functional characteristics.

[1]Center for Gastrointestinal Biology and Disease (CGIBD), Department of Medicine, Division of Gastroenterology and Hepatology, School of Medicine, UNC Microbiome Core, University of North Carolina, Chapel Hill, NC, USA. [2]Department of Plant and Microbial Biology, North Carolina State University, Raleigh, NC, USA. [3]Department of Botany and Microbiology, College of Science, King Saud University, Riyadh, Saudi Arabia. [4]Department of Psychological Sciences, Kent State University, Kent, OH, USA.
*Corresponding author. Email: azcarate@med.unc.edu

This review aims to discuss the existent literature focused on microbial dipeptidyl peptidase 4 (mDPP-4) of the S9B and S15 families, including DPP-4 and PepX (Xaa-Pro dipeptidyl peptidase), respectively, and their potential role as HMIs in the mammal gastrointestinal tract. The focus on the peptidase families S9 (B subfamily) and S15 within the current literature review is supported by critical biochemical and structural similarities that these enzymes share with hDPP-4, asserting their potential role as HMIs in the mammalian gastrointestinal tract. The S9B and S15 peptidases not only mirror the enzymatic specificity of hDPP-4 in their preference for X-Pro dipeptides but also showcase a nearly identical conservation of the catalytic triad, affirming their parallelism in enzymatic activity. Further, the structural homology is evident in the crystallography and AlphaFold structures of the S9B family, which are similar to that of hDPP-4, a strong predictor of functional similarity, further supporting the rationale for their study in the context of HMIs.

GUT MICROBIAL SERINE PEPTIDASES

Proteases hold notable applications in physiological, biomedical, and commercial fields. Their diverse and versatile nature makes them crucial in numerous biological processes, including metabolism, cell signaling, and protein regulation. Furthermore, their potential applications in food processing, pharmaceuticals, and biotechnology make them attractive to researchers and the industry. Serine peptidases, with 10 clans, more than 54 families, and 21 subfamilies, are the most prevalent class of proteolytic enzymes (*7*). While earlier studies primarily centered on human proteolytic activity in disorders such as inflammatory bowel disease (IBD) and irritable bowel syndrome (IBS), there is now an increasing recognition of the substantial contributions made by microbial serine peptidases within the gut ecosystem (*9*, *10*). For instance, increased serine proteolytic activity has been found in the stool supernatants of patients with IBD and IBS, compared to those of healthy individuals, which could originate from both the host and gut microbes (*11*, *12*).

The best-characterized bacterial serine peptidases belong to the S1B, S1C, S6, S8A, S9B, S14, and S15 families. The S9 family, first described in 1991 by Rawlings *et al.* (*13*), includes protein sequences from the subfamily S9B and were determined to be homologous to those of DPP-4 of *Homo sapiens*, which was identified earlier in 1966 (*14*). hDPP-4 has been the focus of extensive research across a range of eukaryotic biological functions, including cell adhesion, HIV infection, apoptosis, and the regulation of tumorigenicity in melanoma cells and T cell activation. One of the most critical functions of hDPP-4 is its role in the degradation of the incretin hormones such as glucagon-like peptide-1 (GLP-1). This activity is particularly notable in the context of diabetes research and treatment (*15*–*17*). Like S9B, S15 peptidases specialize in cleaving Xaa-Pro dipeptides from the N terminus of peptide chains, but with a strong preference for proline at position P1. This specificity is critical in the processing of peptides like glucagon and GLP-1, where proline and other amino acids such as alanine and glycine can be near the N terminus (*18*, *19*). Traditionally, the family S15 has been studied within the proteolytic system of the lactic acid bacteria (LAB) group to hydrolyze peptides derived from β-casein in fermented milk products sequentially (*20*, *21*). However, recent advancements have led to exploring the potential of engineering S15 peptidases for the specific cleavage of glucagon and GLP-1 from fusion proteins (*19*).

The targeted investigation of particular serine peptidase families, especially S9B and S15, is gaining substantial attention in light of their similarity in structure and function to hDPP-4 (*6*, *22*, *23*). This suggests that these microbial enzymes could be analogous in function and possibly in therapeutic targeting. Experimental evidence has shown that both S9B and S15 peptidases cleave a wide range of the same substrates as hDPP-4 and are inhibited by gliptin-class DPP-4 inhibitors (DPP-4is) (*3*, *6*).

BRIDGING THE GAP BETWEEN MICROBIAL AND hDPP-4: IMPLICATIONS IN HOST HOMEOSTASIS AND THE POTENTIAL CONTRIBUTION OF THE GUT MICROBIOME

Historically, hDPP-4 has been studied for its role in glucose homeostasis and insulin regulation by degrading incretin hormones such as GLP-1 and gastric inhibitory polypeptide (GIP) (*24*). Further evidence suggests that hDPP-4 influences the gut-brain axis, modulating other DPP-4 substrates integral to cognitive processes (*24*, *25*), as demonstrated in clinical evidence and rodent models. For instance, upon cleavage by hDPP-4, neuropeptide Y (NPY) undergoes a reduction in its affinity for Y1 and Y5 receptors, crucial for mediating its effects on stress responses, learning, and memory regulation (*26*, *27*), reducing NPY's anxiolytic effect. Similarly, hDPP-4 (and potentially mDPP-4) cleaves peptide YY (PYY) to produce its shorter form, $PYY_{3\text{-}36}$. While the longer form of PYY can activate both Y1 and Y2 receptors, $PYY_{3\text{-}36}$ shows a preference for the Y2 receptor, and the Y2 receptor has been reported to be linked to PYY appetite-suppressing properties (*28*). Last, hDPP-4 has been shown to cleave substance P (SP), a neuromodulator with neuroprotective properties (*29*), which promotes non-amyloidogenic amyloid-β precursor protein (APP) processing and inhibits the deposition of neurotoxic amyloid-β (Aβ) peptides, possibly by regulating voltage-gated potassium channel currents implicated in Aβ-induced neuronal apoptosis (*30*). In a rat model, DPP-4 cleaved SP, producing the truncated forms $SP_{3\text{-}11}$ and $SP_{5\text{-}11}$ (*31*). SP plays a crucial role in pain transmission and its association with neuroinflammation could contribute to cognitive impairments (*32*).

mDPP-4, which belongs to the S9B subfamily, shares structural similarities with the S9A subfamily (*33*) yet shares closer functional similarities in substrate specificity with the S15 subfamily peptidases, despite structural differences. The S15 subfamily peptidase, PepX, has been suggested to function as a complement to mDPP-4. Although, taxonomic assessment of the sequence-based phylogenetic relationship between the S9 and S15 peptidase families reveals that, beyond the catalytic triad, structural and localization differences are evident. Olivares *et al.* (*34*) showed that the two microbial protease families have distinct evolutionary origins, arising independently from separate bacterial lineages. The S9 family, characterized by the conserved GWSPGGF motif, shows closer alignment with eukaryotic DPP-4 proteins than the S15 family. Moreover, differences in cellular localization between the protease families have been observed as the mDPP-4 enzyme has a 23–amino acid N-terminal signal peptide indicative of extracellular localization, whereas S15 peptidases are predominantly intracellular (*34*).

In a recent study, screening extracts for enzyme activity from 10 mixed fecal cultures of healthy humans cultured in brain heart infusion medium supplemented with fluorometholone identified mDPP-4 as the enzyme with the most pronounced HMI activity, emphasizing a potential influence on host physiology and regulation (*3*). mDPP-4

role has been studied in peptide metabolism and lignocellulose digestion in ruminants (*35*) and implicated in periodontal disease and biofilm formation in the oral cavity (*36*) and, more recently, as an antidiabetic target (*3*). mDPP-4 has been reported in Gram-negative bacteria, specifically in species of *Prevotella* and *Bacteroides* (*3*, *23*, *37*). Coincidentally, the gut microbiome composition in patients with type 2 diabetes mellitus (T2DM), compared to that in nondiabetic individuals, showed a significant positive correlation between the ratios of the *Bacteroides-Prevotella* group and the *Clostridium coccoides–Eubacterium rectale* group with plasma glucose levels, independent of individual body mass index (BMI) (*38*). Additionally, another study on patients with obesity undergoing bariatric surgery found that the family *Prevotellaceae* was significantly more abundant in patients with obesity (*39*). Further, the microbiome composition of individuals newly diagnosed with unmedicated Alzheimer's disease (AD) and mild cognitive impairment (MCI) has been characterized by changes in the relative abundance of *Bacteroides* and *Prevotella* (*40*, *41*). Moreover, these genera have been implicated in other neurocognitive disorders, including major depressive disorder (*42*), cognitive impairment due to minimal hepatic encephalopathy (*43*), and autism (*44*). Despite extensive documentation of shifts in taxa with mDPP-4 activity within the gut microbiome, investigations into its implications for human cognitive, metabolic, and systemic health still need to be conducted.

THE STRUCTURE AND CHARACTERISTICS OF THE hDPP-4

hDPP-4 (also known as lymphocyte cell surface protein CD26, EC 3.4.14.5) is a multifunctional serine protease that plays a pivotal role in various activities of the human body. Eukaryotic DPP-4 was first identified in rat liver in the early 1960s by Hopsu-Havu and Glenner (*14*) and initially named glycyl proline naphthylamidase. The enzyme's Gly-Pro sequence, reminiscent of sequences found in collagens, prompted speculation regarding its potential role in collagen metabolism. Yet, DPP-4 does not cleave Pro-Pro or Pro-Hyp bonds, commonly associated with the Gly-Pro sequence in collagen. This distinction initially complicated the understanding of hDPP-4's precise physiological role (*45*). Its physiological relevance in regulating incretin hormones was not established until the late 20th century (*46*), and it is now well-established that hDPP-4 is involved in many enzymatic and nonenzymatic functions (*47*, *48*). One of its main enzymatic functions is to cleave off N-terminal dipeptides with either L-proline or L-alanine at the penultimate position interacting with various binding partners, such as adenosine deaminase and caveolin-1 (*48*–*52*). hDPP-4 has multiple nonenzymatic functions, including involvement in nutrition, nociception, cell adhesion, psych-neuroendocrine regulation, immune response, and cardiovascular adaptation (*48*, *53*, *54*). A recent investigation into hDPP-4 nonenzymatic role in patients with T2DM and db/db mice model of diabetes revealed that increased nonenzymatic DPP-4 activity correlated with cognitive impairment, potentially by targeting PAR2 in the hippocampus, leading to mitochondrial dysfunction through a glycogen synthase kinase 3β–mediated mechanism (*47*). In mammals, DPP-4 can be found in two primary forms: a membrane-anchored cell surface peptidase on an array of cells, including lymphocytes, endothelial, and epithelial cells, and in soluble form circulating in the blood (*48*, *55*).

The hDPP-4 is a homodimer and a type II transmembrane glycoprotein. It comprises a six–amino acid cytoplasmic tail, a 22–amino acid transmembrane domain, and a large 738–amino acid extracellular domain (*56*, *57*). The extracellular domain includes a flexible stalk, a glycosylation-rich region, a cysteine-rich region, and a catalytic site responsible for cleaving dipeptides from the N terminus of target peptides (*48*, *58*). The crystal structure of hDPP-4 reveals a unique α/β hydrolase fold featuring a central eight-stranded β sheet surrounded by α helices (*59*). This fold contributes to the enzyme's stability and substrate recognition, with most monoclonal anti–DPP-4 antibodies targeting the glycosylation-rich and cysteine-rich region domains. The active site, crucial for catalytic function, is formed by a catalytic triad composed of serine, aspartate, and histidine residues (Ser^{630}, Asp^{708}, and His^{740}). Two identified openings for the active site include a side opening and a propeller tunnel. Studies have shown that NPY is cleaved by hDPP-4 through the side opening. These residues in the catalytic triad work together to facilitate the nucleophilic cleavage of the peptide bond of substrates, enabling the cleavage of dipeptides from the N terminus (*48*, *59*).

In addition to its unique fold and catalytic triad, hDPP-4 has an extensive substrate-binding pocket that recognizes various substrates. This feature, combined with its structural flexibility, contributes to the enzyme's broad substrate specificity and ability to process a wide range of substrates, including those involved in cognitive and neural processes (*60*). Thus, understanding the structural and functional characteristics of DPP-4 originating from multiple organisms is critical for developing targeted therapeutic strategies and designing inhibitors that modulate its activity to influence host cognitive function.

THE mDPP-4

mDPP-4 is an S9B peptidase. According to the MEROPS database (www.ebi.ac.uk/merops/index.shtml), the S9 family contains various serine-dependent peptidases. This family of peptidases was reported in 1991 (*13*) when Rawlings and collaborators realized that a newly determined prolyl oligopeptidase from the pig brain was homologous to those of hDPP-4 and acylamino acyl-peptidase. These examples represent a subfamily type (S9A, S9B, and S9C). Recently, a new subfamily (S9D) was added, which includes a glutamyl endopeptidase.

Early studies showed that the DPP-4 enzyme was highly conserved across eukaryotic and prokaryotic organisms, with their amino acid sequences displaying notable similarity in structure and function, leading to their classification under the same Enzyme Commission number (EC 3.4.14.5) (*6*, *22*, *23*). mDPP-4 was first reported in DPP-4 knockout (KO) mice, as residual DPP-4 activity was detected in their plasma (*61*), sparking speculations that it might stem from the gut microbiota and the possibility of mDPP-4 translocating from the microbiota across the blood-brain barrier. A subsequent study by Olivares *et al.* (*6*) in germ-free and colonized gnotobiotic mice showed significantly increased DPP-4 activity in the cecal content of colonized mice, with no increased *dpp-4* gene expression in the mouse cecal tissue. The observed increase was credited to mDPP-4 activity derived from the gut microbiome (*6*).

mDPP-4 has been reported in Gram-negative bacteria, including *Prevotella ruminicola*, *Prevotella albensis*, *Prevotella pallens*, *Prevotella nigrescens*, *Prevotella intermedia*, *Prevotella aurantiaca*, *Bacteroides thetaiotaomicron*, *Bacteroides xylanisolvens*, *Bacteroides fragilis*, *Bacteroides eggerthii*, *Bacteroides vulgatus*, and *Bacteroides dorei* (*3*, *23*, *37*, *62*). mDPP-4 has also been studied in periodontopathic bacteria, such as *Porphyromonas gingivalis* and *Tannerella forsythia* (*62*). Further, studies have reported mDPP-4 activity in fungi, including its role in the

opportunistic colonization of lung tissue by *Blastomyces dermatitidis* (*63*) and as a fungal virulence factor in dermatophytes such as *Microsporum canis* (*64*, *65*) and *Trichophyton rubrum* (*66*). Like soluble hDPP-4, a recent study examining bacterial extracellular vesicles (BEVs) produced by *B. thetaiotaomicron* showed that these BEVs were enriched in 113 proteins and enzymes, including mDPP-4 (*67*). This observation suggested that mDPP-4 in BEVs could be equivalent to soluble hDPP-4.

The structure of mDPP-4 has been studied in several bacterial species, including *P. gingivalis* (*68*), *Stenotrophomonas maltophilia* (*69*), and *Pseudoxanthomonas mexicana* WO24 (*70*) and *Hoylesella loescheii* (formerly known as *Prevotella loescheii*) American Type Culture Collection 15930 (*71*) (listed in Table 1). mDPP-4, in conjunction with other peptidases, plays a crucial nutritional function in asaccharolytic bacteria, which relies on proteins or peptides for energy, transporting cleaved dipeptides across the plasma membrane using a proton-dependent oligopeptide transporter (*70*, *72*, *73*). The first ever reported mDPP-4 crystal structure (from *S. maltophilia*) reported modifications around the active site compared to hDPP-4, specifically in the Arg^{125} residue, which is involved in the recognition of a substrate carbonyl group in hDPP-4 and is absent in *S. maltophilia*'s mDPP-4 (Fig. 1) (*69*). Conversely, the mDPP-4 crystal structure from *P. gingivalis* showed that the substrate-binding site of *P. gingivalis* was similar to the active site of hDPP-4 where the Arg^{125} residue was equivalent to Arg^{115} in *P. gingivalis* (*68*). Other dipeptides revealed high similarity to the *S. maltophilia* DPP-4, and the complexed structures demonstrated the presence of an acyl-enzyme intermediate in the active site instead of the tetrahedral intermediates reported in hDPP-4, indicating a novel substrate recognition mechanism of mDPP-4 s (*70*). Last, a

Table 1. mDPP-4 from different strains and its similarity to hDPP-4. NCBI, National Center for Biotechnology Information; PDB, Protein Data Bank.

Species	Isolate type	hDPP-4 analog name	Structure type	RMSD*	Amino acid sequence similarity*	Catalytic triad residues†	Protein accession number
B. thetaiotaomicron	Reference sequences	Dipeptidyl peptidase IV	Crystal structures: dimer	7403 Atoms at 2.335 Å	44.3%	Ser^{606}, Asp^{681}, and His^{713}	PDB: 7Y4F (1.92 Å)
			Crystal structures: monomer	3580 Atoms at 1.727 Å			
P. mexicana	Reference sequences	Dipeptidyl peptidase IV	Crystal structures: dimer	7705 Atoms at 3.685 Å	38.7%	Ser^{613}, Asp^{689}, and His^{721}	PDB: 5YP1 (2.47 Å)
			Crystal structures: monomer	3606 Atoms at 2.257 Å			
P. gingivalis	Reference sequences	Dipeptidyl peptidase IV	Crystal structures: dimer	7106 Atoms at 1.739 Å	43.6%	Ser^{593}, Asp^{668}, and His^{700}	PDB: 5OLJ (2.20 Å)
			Crystal structures: monomer	3503 Atoms at 1.740 Å			
S. maltophilia	Reference sequences	Dipeptidyl aminopeptidase IV	Crystal structures: dimer	7322 Atoms at 2.341 Å	37.3%	Ser^{610}, Asp^{685}, and His^{717}	PDB: 2ECF (2.80 Å)
			Crystal structures: monomer	3615 Atoms at 2.402 Å			
Bacteroides ovatus	Reference sequences	Dipeptidyl aminopeptidase IV	Crystal structures: dimer	6411 Atoms at 2.271 Å	39.5%	Ser^{603}, Asp^{678}, and His^{710}	PDB: 4Q1V (2.48 Å)
S. copri	Metadata from (*41*)	Peptidase S9-2	Predicted structures: monomer (AlphaFold2)	3697 Atoms at 2.048 Å	43.1%	Ser^{583}, Asp^{657}, and His^{689}	NCBI Reference Sequence: WP_153073221.1
		Prolyl-tripeptidyl-peptidase		3067 Atoms at 1.795 Å	37.1%	Ser^{645}, Asp^{720}, and His^{752}	GenBank: MBW0040962.1
H. loescheii	Reference sequences	DPP IV N-terminal domain–containing protein	Predicted structures: monomer (AlphaFold2)	3192 Atoms at 1.899 Å	37.4%	Ser^{598}, Asp^{673}, and His^{705}	NCBI Reference Sequence: WP_281643346.1
P. albensis	Reference sequences	Dipeptidyl peptidase IV	Predicted structures: monomer (AlphaFold2)	3839 atoms at 2.198 Å	43.5%	Ser^{600}, Asp^{674}, and His^{706}	GenBank: CAC42932.1
Prevotella dentalis	Reference sequences	DPP IV N-terminal domain–containing protein	Predicted structures: monomer (AlphaFold2)	3118 Atoms at 1.866 Å	38.6%	Ser^{609}, Asp^{684}, and His^{716}	NCBI Reference Sequence: WP_005846480.1
P. ruminicola	Reference sequences	Prolyl tripeptidyl peptidase	Predicted structures: monomer (AlphaFold2)	3054 Atoms at 2.009 Å	42.2%	Ser^{576}, Asp^{651}, and His^{683}	GenBank: GJG34220.1

*Aligned to *H. sapiens* (Hs DPP-4). †Corresponding to Ser^{630}, Asp^{708}, and His^{740} in *H. sapiens*.

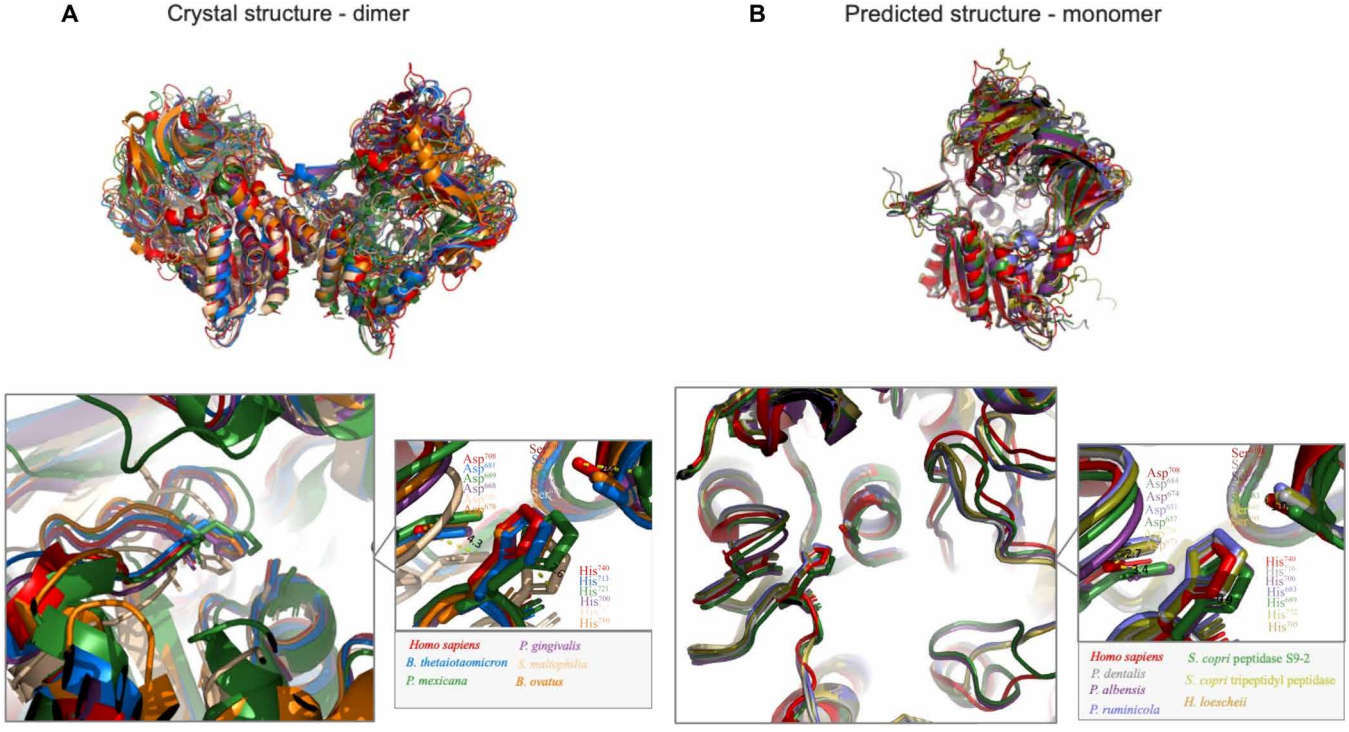

Fig. 1. mDPP-4 structures from reported strains. (**A**) Superimposed dimer crystal structures. Inset close-up of the catalytic triad showing that the catalytic triad is largely conserved throughout all structures. Distances indicated: *H. sapiens* Ser^{630} gamma oxygen to *P. mexicana* Ser^{610} gamma oxygen, 2.4 Å; *H. sapiens* Asp^{708} delta 1 oxygen to *S. maltophilia* Asp^{685} delta 2 oxygen, 4.3 Å; and *H. sapiens* His^{740} delta 2 carbon to *S. maltophilia* His^{717} delta 2 carbon, 2.65 Å. (**B**) Predicted AlphaFold2 monomer structures superimposed with close-up of the catalytic triad showing that the catalytic triad is again largely conserved throughout all structures. Distances indicated: *H. sapiens* Ser^{630} gamma oxygen to *S. copri* peptidase S9-2 Ser^{583} gamma oxygen, 1.0 Å; *H. sapiens* Asp^{708} delta 2 oxygen to *P. ruminicola* Asp^{651} delta 2 oxygen, 2.7 Å; *H. sapiens* Asp^{708} delta 2 oxygen to *P. albensis* Asp^{674} delta 2 oxygen, 3.4 Å; and *H. sapiens* His^{740} delta 2 carbon to *S. copri* peptidase S9-2 His^{689} delta 2 carbon, 0.9 Å. The color-coded catalytic triad, depicted in the close-ups, corresponds to the different species indicated below the catalytic triad close-up.

study examining the crystal structure of mDPP-4 from *P. mexicana* WO24 complexed with several hDPP-4 inhibitors (hDPP-4is) showed significantly weaker inhibitory activities than hDPP-4 (*70*).

Recent research indicates that mDPP-4 enzymes play a crucial role in the physiology of gut-associated bacteria, affecting both the individual fitness of bacterial species and the dynamics of complex microbial communities. Keller *et al.* (*74*) showed that BtDPP-4 is critical for maintaining the integrity of the bacterial envelope and enhancing *B. thetaiotaomicron* resilience within diverse microbial communities. They found that deletion of BtDPP-4 resulted in increased sensitivity to cell envelope stressors such as vancomycin, deoxycholic acid, and polymyxin B. They also demonstrated that the activity of BtDPP-4 is not solely dependent on its proteolytic function; using a catalytically inactive mutant of BtDPP-4 retained the ability to rescue phenotypes related to cell integrity compared to deletion mutants and restored its resistance to tested stressors. These results indicate that BtDPP-4 has an important noncatalytic role, likely contributing to the integrity of the periplasmic space through protein-protein interactions or a scaffolding function. Furthermore, BtDPP-4 was observed to enhance the competitive capabilities of *B. thetaiotaomicron* in vitro within a consortium of 14 stool-derived gut commensal species, including *B. fragilis*, *B. vulgatus*, *Faecalibacterium prausnitzii*, and *Escherichia coli*. Notably, similar to its effects on cell integrity, this enhancement did not rely on proteolytic activity but rather likely engaged in protein-protein interactions. These results underscore the importance of BtDPP-4 beyond its enzymatic activities, emphasizing its role in the maintenance of cellular stability and facilitation of community integration. These findings warrant further investigation into analogous functions in other microbial species to elucidate the mechanisms by which gut microbiota influence host health (*74*).

The potential impact of mDPP-4 on host health has been highlighted by a study that showed that a higher abundance of *P. gingivalis* in chronic periodontitis was associated with AD (*75*). *P. gingivalis*, known for its acid-resistant properties, has been shown to survive the harsh pH conditions of the stomach. This resilience allows it to persist where individuals with periodontitis swallow 10^8 to 10^{10} of these bacteria daily, introducing them into the gastrointestinal tract (*76*–*80*). The mDPP-4 encoded by *P. gingivalis* was able to degrade incretin peptides, similar to the mammalian DPP-4 in a mouse model, resulting in an increased and prolonged postprandial hyperglycemia leading to insulin resistance (IR) (*62*). Another *P. gingivalis* mDPP-4–like enzyme, DPP-7, had more potent hydrolyzing activity against incretin hormones and other gastrointestinal peptides (*73*). The intravenous administration of mDPP-7 with oral glucose decreased plasma insulin and GLP-1 levels in mice. Synthetic fluorogenic peptide analysis revealed that the reaction rate (k_{cat}) was significantly elevated in the P1′ and P2′ residues for *P. gingivalis* mDPP-7 (*73*).

Similarly, compromised permeability, frequently witnessed with aging, obesity, and chronic illnesses, hints at the intestinal translocation

of mDPP-4 (*81*, *82*). A study evaluating the fasting serum levels of active GLP-1 and soluble hDPP-4 in patients with average weight (BMI ≤ 18.5 and ≥ 22.9 kg/m^2), overweight (BMI ≤ 23 and ≥ 27.4 kg/m^2), and obesity (BMI ≥ 27.5 kg/m^2) showed that levels of soluble DPP-4 were significantly elevated in patients with obesity, registering at 13.5%, in comparison to the 2.7% observed in normal weight individuals. These were negatively correlated with active GLP-1 and positively associated with IR (*83*). Given that obesity is associated with low-grade inflammation and compromised intestinal permeability (*84*), this could be a mechanism through which mDPP-4 influences the overall levels of active DPP-4 and GLP-1. This hypothesis is supported by a recent mouse study in which *E. coli* Nissle 1917 (EcN) carrying the *Bacteroides* mDPP-4 gene was orally administered to mice fed a high-fat diet (HFD) or treated with dextran sulfate sodium/indomethacin (as leaky gut models with compromised barrier functions) and control EcN group. mDPP-4 decreased active GLP-1 activity and disrupted glucose homeostasis, suggesting a compromised gut barrier, while GLP-1 activity was not affected in control mice (*3*).

A study aimed to investigate the impact of the human gut microbiome on the serum metabolome and its association with IR of 277 nondiabetic individuals identified *Prevotella copri* and *B. vulgatus* as potentially inducing IR using the homeostatic model assessment (HOMA), a method used to quantify IR and β cell function based on fasting glucose and insulin levels where a higher HOMA score indicates increased IR. Both species were positively correlated with HOMA-IR and were associated with aggravating glucose intolerance (*85*). To address a potential relation between *S. copri* and abnormal glucose metabolism, the authors administered *S. copri* (5×10^8 colony-forming units) twice weekly to C57BL/6J male mice fed a HFD for 2 weeks. The *S. copri* group had worse glucose tolerance and increased serum total branched-chain amino acid levels (*85*). Although the authors did not assess GLP-1 or DPP-4 in the study, these findings aligned with the broader body of research on this topic. Highlighting the complexity of glucose homeostasis, mice fed a high-fiber, low-fat diet reported contrasting outcomes regarding the relationship between glucose intolerance and the *S. copri* challenge (*86*).

Consistent with the study by Pedersen *et al.* (*85*), we reported that a random forest analysis of microbiomes at baseline showed that *P. ruminicola*, *B. thetaiotaomicron*, and *B. xylanisolvens* were associated with impaired cognitive status in middle-aged and older adults (*41*). The participants that received a probiotic supplementation for 12 weeks had a reduced abundance of *Prevotella* and more stable hemoglobin A1C (HbA1c) levels compared to an increase in placebo controls (*41*, *87*). This microbial shift corresponded with cognitive improvements in individuals with MCI (*88*). *P. ruminicola*, *B. thetaiotaomicron*, and *B. xylanisolvens* have been reported recently to have high mDPP-4 activity (*3*, *35*). These findings collectively suggest that mDPP-4 plays a significant role in the host's physical and physiological health. However, further research is needed to directly distinguish the extent to which mDPP-4 activity influences host health.

DPP-4is AND THEIR METABOLIC IMPACT

DPP-4is are important therapeutic options for managing T2DM (*25*, *89*). In the late 20th century, the discovery of the incretin effect revealed that gut-derived incretin hormones (GLP-1 and GIP) could stimulate insulin secretion in response to oral glucose (*89*). Incretin hormones have a short half-life due to the hDPP-4–mediated rapid degradation (*90*). This discovery spurred research into developing drugs that could inhibit DPP-4 to enhance the incretin effect and improve glycemic control in patients with T2DM (*16*, *91*). The first DPP-4i, sitagliptin, was approved by the US Food and Drug Administration (FDA) in 2006 (*92*). Since then, other DPP-4i, including vildagliptin, saxagliptin, linagliptin, and alogliptin, have been introduced into clinical practice (*93*). DPP-4is have demonstrated additional therapeutic potential beyond T2DM, affecting conditions such as cardiovascular diseases (*94*), chronic kidney diseases (*95*), intestinal injury (*96*), atherosclerosis (*97*), inflammatory disorders (*98*), and neurodegenerative disorders (*99*). hDPP-4is have been shown to stimulate intestinal epithelial growth through GLP-2 and modulate the immune response and intestinal inflammation via the interleukin-6/Janus kinase 2/signal transducer and activator of transcription 3, HMG box 1/RAGE/nuclear factor κB, and Nrf2/HO-1 pathways (*100*).

T2DM is a risk factor for cognitive dysfunction and progression to dementia (*101*). Studies have linked T2DM to neurodegenerative diseases and cognitive impairment, with a twofold higher risk of AD for patients with prediabetes and T2DM (*102*). Similar results have been found in patients with Parkinson's disease (PD) (*103*), Huntington's disease (*104*), and vascular dementia (*25*, *105*). Given the increased risk of cognitive decline and dementia associated with diabetes, hDPP-4is have gained interest for their potential role in neurodegenerative diseases and cognitive functions, particularly with peptides such as NPY, PYY, and GLP-1 via peptide inactivation or modification in receptor preference (*25*, *28*, *106*). In the triple-transgenic mouse model of Alzheimer's disease (3xTg-AD) mouse model, the hDPP-4i linagliptin reversed cognitive deficit, reduced neuroinflammation, and improved brain incretin levels of GLP-1 and GIP; notably, despite the influence of plasma incretins on glucose levels, these changes brain incretin levels did not affect plasma glucose levels (*107*). Additionally, experimental and clinical findings emphasized NPY's significance in cerebral function, particularly in regulating anxiety, mood, and cognition via its unique receptor affinity to Y1, Y2, and Y5 receptors (*27*, *28*, *108*, *109*). Borzì *et al.* (*110*) conducted a clinical study to evaluate a commercially available hDPP-4i (sitagliptin) in older adults with diabetes, with or without MCI or AD. Patients receiving DPP-4i for 6 months had improved cognitive function, demonstrated by higher minimental state examination (MMSE) scores compared to the metformin group. Further, a retrospective study found that vildagliptin, a DPP-4i, may protect cognitive functioning in elderly patients with diabetes with MCI demonstrated by maintaining their MMSE score (*110*).

Recent studies suggested that specific classes of hDPP-4i can reverse cognitive dysfunction. In diabetic rats, saxagliptin alleviated cognitive dysfunction through anti-inflammatory and anti-tau phosphorylation mechanisms (*111*). Another study demonstrated that Gramcyclin A reversed cognitive decline, reduced Aβ plaques, decreased neuroinflammation, and enhanced brain glucose uptake in APP/PS1/tau-triple transgenic mice (*112*). Kosaraju *et al.* (*107*) showed that the administration of linagliptin in a mouse model of AD (3xTg-AD) improved cognitive impairment, decreased neuroinflammation, and increased levels of the brain incretin hormones GLP-1 and GIP while not affecting plasma glucose levels. The authors reported similar findings when using vildagliptin (*107*). hDPP-4i has shown evidence expanding beyond diabetes management to show potential in treating a broader spectrum of conditions, including

renal, inflammatory diseases, and neurological conditions, including cognitive decline.

hDPP-4i impact on gut microbiome composition

There is a growing interest in the correlation between DPP-4i and gut microbiome dynamics (*113*, *114*). A study of 70 Korean patients with T2DM revealed that an intervention with gemigliptin and metformin led to changes in the gut microbiome, characterized by an increased abundance of *Bacteroides* and a significant depletion of taxa belonging to *Bacillota*, including *Lactobacillus*, *Ruminococcus torques*, and *Streptococcus* (*113*). Conversely, in a 12-week randomized, double-blind study of 51 adults with T2DM who received either sitagliptin (a DPP-4i), liraglutide [a GLP-1 receptor (GLP-1R) agonist], or placebo, there were no distinct alterations to the gut microbiota, despite sitagliptin and liraglutide's effects on glucose metabolism and bile acid profiles. The lack of changes in the gut microbiome might be explained by the fact that participants in this study had been on metformin and sulfonylurea (SU) derivatives for at least 3 months. Metformin has been shown to affect the composition of the gut microbiome either on monotherapy or combined with SU (*115*). In human studies, particularly in patients with T2DM and obesity, metformin increased the relative abundance of *Akkermansia*, specifically *Akkermansia muciniphila*, and *Enterobacteriaceae*, specifically of the genus *Escherichia* (*116*–*119*). A metformin intervention in C57BL/6 mice resulted in a significant increase in the relative abundance of phyla *Bacteroidota* and *Verrucomicrobia*, and genera *Akkermansia* and *Bacteroides* (*120*, *121*). Rat studies similarly noted a shift in the relative abundance of *Akkermansia* following metformin treatment (*122*–*124*).

There are no human studies on the effect of DPP-4i alone on the gut microbiome. In animal models, a study showed that C57BL/6 mice colonized with stools from patients with T2DM and fed a HFD showed a significantly altered gut microbiota composition after DPP-4i saxagliptin administration. Key findings included an increased abundance of beneficial bacteria like *Akkermansia* and *Lactobacillus*, known for maintaining the integrity of the gut barrier function and modulating inflammation (*125*–*128*). The compositional changes promoted a functional shift correlated with increased succinate production, improved glucose metabolism, lower levels of inflammation, and enhanced insulin sensitivity. Notably, such changes were not observed in the mice group receiving α-glucosidase inhibitor treatment, suggesting a distinctive mechanism involving the gut microbiome (*114*). In diabetic rats, sitagliptin altered the gut microbiome composition at the phylum level, increasing *Bacteroidota* and *Pseudomonadota* while reducing *Bacillota* and specifically increasing the abundance of *Roseburia* and reducing *Blautia* at the genus level (*129*). The baseline microbiome of the animals was characterized by a reduced relative abundance of beneficial species like *Lactobacillus* and *Bifidobacterium*. However, following a sitagliptin intervention, the relative abundance of *Bifidobacterium* was increased. Likewise, in the streptozotocin-induced TSDM rat model, the administration of sitagliptin resulted in a significant decrease in the relative abundance of *Bacteroidota* and an increase in *Bacillota*. At the genus level, *Clostridium*, *Fusobacterium*, *Lactobacillus*, and *Streptococcus* were significantly higher in sitagliptin-treated rats. Further, these changes in rats were associated with less neuronal damage by ameliorating changes of pyramidal neurons in rats' hippocampus (*130*). Last, mice fed an HFD and treated with saxagliptin had a decreased relative abundance of *Bacteroides* and *Prevotella* (*131*).

Do hDPP-4i affect mDPP-4?

While gliptins (hDPP-4i) have become fundamental to managing T2DM, emerging evidence suggests a reduced response in some patients (*132*, *133*). A recent study reported significant variations in HbA1c levels in 57 patients newly diagnosed with T2DM after a 3-month sitagliptin intervention. The low-response patients (patients who did not achieve an HbA1c reduction to ≤6.5% or a decrease of >1% during sitagliptin treatment) had significantly higher DPP-4 activity (*3*). The study showed through crystal structures of the mDPP-4 from *Bacteroides* sp. in complex with sitagliptin that the E342 side chain forced a significantly different conformation than what was observed in the hDPP-4 conformation. The trifluoromethyl triazolopyrazinyl group in sitagliptin was rotated ~40° from the hDPP-4 conformation, leading to the formation of a less stable interaction and providing a potential explanation for why the drug was ineffective in inhibiting mDPP-4 (Fig. 2). The authors identified a derivate of daurisoline (Dau-d4) as a novel, selective inhibitor of mDPP-4 and demonstrated that Dau-d4 decreased mDPP-4 activity in mouse feces and extraluminal intestinal tissue without influencing intestinal host DPP-4 levels. Dau-d4 also improved glucose homeostasis and was associated with higher levels of active GLP-1 in plasma (*3*). Another animal study showed that vildagliptin decreased overall DPP-4 activity in feces and cecal content, positively influenced the gut microbiota composition, and maintained intestinal homeostasis in mice fed a Western diet, primarily by reducing *Oscillibacter* while increasing *Lactobacillus* and propionate levels (*134*). Propionate and acetate have been shown to stimulate GLP-1 secretion via the G protein–coupled free fatty acid receptor 2 (FFAR2) and FFAR3 in primary murine colonic cultures (*135*).

While the mDPP-4 from periodontopathic bacterial strains was effectively inhibited by sitagliptin, vildagliptin, and P32/98 (*62*), sitagliptin, linagliptin, teneligliptin, gemigliptin, and trelagliptin did not inhibit *B. thetaiotaomicron* mDPP-4 activity (*3*). Furthermore, in animal model studies, sitagliptin increased the relative abundance of *Bacteroidetes*, whereas saxagliptin decreased it (*129*, *131*). Using in vitro stool-derived microbial communities, Keller *et al.* (*74*) evaluated the relative abundance and half maximal inhibitory concentration (IC_{50}) of *B. thetaiotaomicron* and BtDPP-4 when treated with hDPP-4i including talabostat, saxagliptin, sitagliptin, and linagliptin. Results demonstrate that talabostat and saxagliptin effectively inhibited BtDPP-4 activity in *B. thetaiotaomicron*, with IC_{50} values comparable to that of hDPP-4. In addition, talabostat and saxagliptin can potentially affect bacterial fitness and the ability of *B. thetaiotaomicron* to thrive in a mixed bacterial community, thereby affecting its role in carbohydrate digestion, microbial balance, and the overall dynamics of the gut ecosystem (*74*).

A recent computational study using molecular dynamics simulations, including mDPP-4 structures from *Segatella copri*, *Phocaeicola vulgatus*, *Bacteroides uniformis*, *Parabacteroides merdae*, and *Alistipes* sp., demonstrated that the predicted mDPP-4 structures exhibited binding scores to tested gliptins similar to those of hDPP-4, suggesting a potential interaction between these drugs and the gut microbiota. One potential concern, however, is that the molecular dynamics simulations were run for a relatively short duration of 200 ns, therefore extending the simulation time in future studies could provide a more comprehensive understanding of the efficacy of the tested hDPP-4is (*136*). Overall, these variations in reports might be attributed to the model organism, the specific DPP-4i, or the baseline microbiome composition differences. Table 2 summarizes available

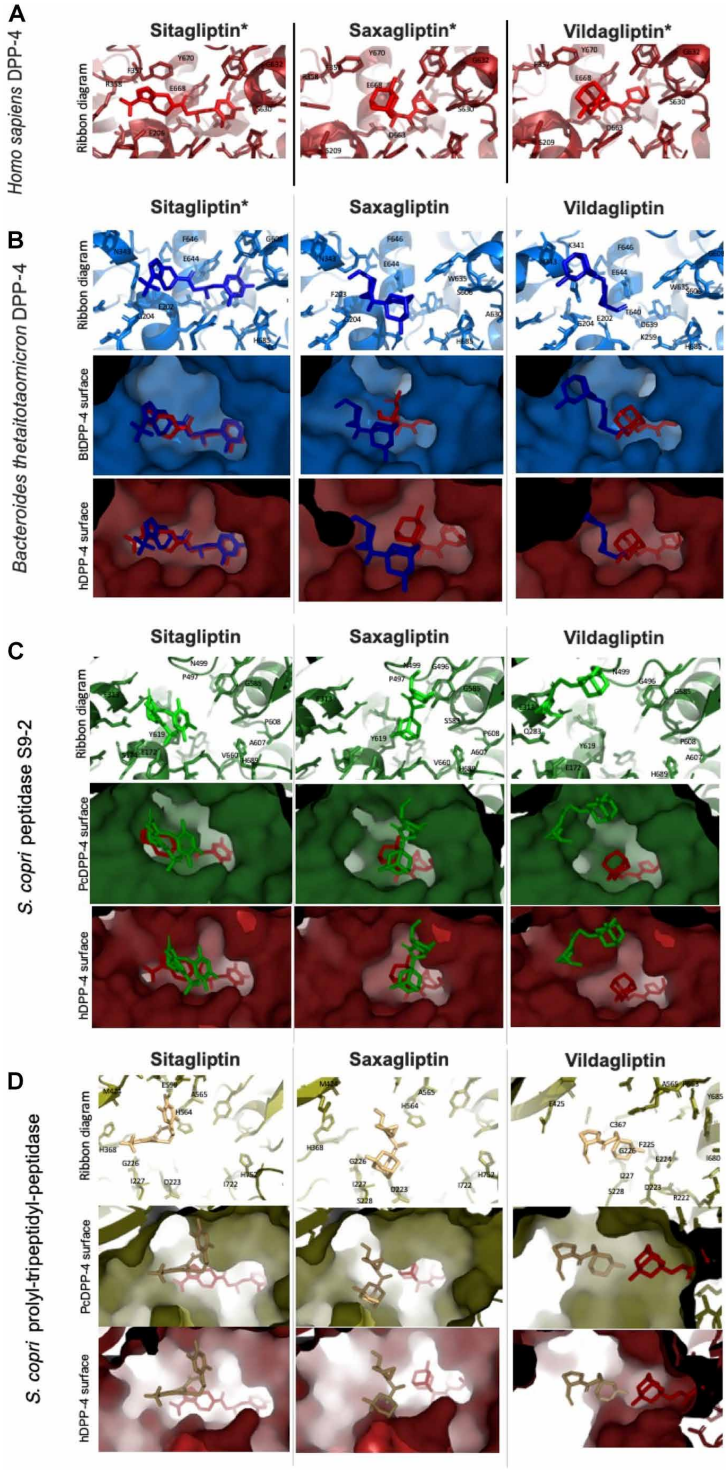

Fig. 2. Interaction between DPP-4is, including sitagliptin, saxagliptin, and vildagliptin, and active site of mDPP-4 from *B. thetaitotaomicron*, *S. copri* peptidase S9-2, and *S. copri* prolyl-tripeptidyl-peptidase. DPP-4 surface colors are red for *H. sapiens*, medium blue for *B. thetaitotaomicron* DPP-4, green for *S. copri* peptidase S9-2, and olive green for *S. copri* prolyl-tripeptidyl-peptidase. DPP-4i colors are bright red for *H. sapiens* confirmation, dark blue for *B. thetaitotaomicron* confirmation, bright green for *S. copri* peptidase S9-2 docking conformation, and orange for *S. copri* prolyl-tripeptidyl-peptidase docking conformation. (**A**) DPP-4i interactions with *H. sapiens* DPP-4: sitagliptin, saxagliptin, and vildagliptin (*) crystal structure conformations. (**B**) DPP-4i interactions with *B. thetaitotaomicron* DPP-4 (BtDPP-4); sitagliptin (*) crystal structure conformation. Saxagliptin and vildagliptin docked with estimated free energy of binding (EFEB) of −2.99 and −2.00 kcal/mol, respectively. (**C**) DPP-4i interactions with *S. copri* peptidase S9-2 (PcDPP-4): sitagliptin, saxagliptin, and vildagliptin docked with EFEB of −0.49, −1.11, and −0.83 kcal/mol, respectively. (**D**) DPP-4i interactions with *S. copri* prolyl-tripeptidyl-peptidase sitagliptin: saxagliptin, and vildagliptin docked with EFEB of −0.87, −1.45, and −1.54 kcal/mol, respectively.

Table 2. Reports of hDPP-4i impact on mDPP-4 and PepX activities. No inhibition, 5 to 0% inhibition; low inhibition, less than 30%; moderate inhibition, between 40 and 70% inhibition; high inhibition, 71 to 94% inhibition; complete inhibition, more than 95% inhibition. ATCC, American Type Culture Collection; PMSF, phenylmethylsulfonyl fluoride.

Peptidase type	Bacterial species	Source	Study Type	Inhibitor/s	Inhibitor/s class	Enzymatic activity	Reference
mDPP-4	*P. gingivalis* (ATCC 33277)	Type strain: periodontopathic bacteria	Monocultures: purified mDPP-4	Sitagliptin, vildagliptin, and isoleucyl thiazolidide (P32/98)	hDPP-4 inhibitors	Complete inhibition	(*62*)
				PMSF	Serine protease inhibitor	Moderate inhibition	
				Leupeptin	Protease inhibitor	Low inhibition	
				EDTA	Chelating agent	Low inhibition	
	T. forsythia (ATCC 43037)	Type strain: periodontopathic bacteria	Monocultures: purified mDPP-4	Sitagliptin, vildagliptin, and isoleucyl thiazolidide (P32/98)	hDPP-4 inhibitors	Complete inhibition	(*62*)
				PMSF	Serine protease inhibitor	Moderate inhibition	
				Leupeptin	Protease inhibitor	Low inhibition	
				EDTA	Chelating agent	Low inhibition	
	P. intermedia (ATCC 25611)	Type strain: periodontopathic bacteria	Monocultures: purified mDPP-4	Sitagliptin, vildagliptin, and isoleucyl thiazolidide (P32/98)	hDPP-4 inhibitors	Complete inhibition	(*62*)
				PMSF	Serine protease inhibitor	Moderate inhibition	
				Leupeptin	Protease inhibitor	Low inhibition	
				EDTA	Chelating agent	Low inhibition	
	B. thetaiotaomicron	Human stool	Monocultures: purified mDPP-4	Sitagliptin, linagliptin, teneligliptin, gemigliptin, and trelagliptin	hDPP-4 inhibitors	Low inhibition	(*3*, *74*)
				Vildagliptin		Complete inhibition	
				Talabostat and saxagliptin		Complete inhibition.	
				Sitagliptin and linagliptin		Low inhibition	
	Oscillibacter valericigenes (DSM18026)	Type strain	Monocultures	Vildagliptin	hDPP-4 inhibitor	Complete inhibition of activity and bacterial growth	(*134*)
	P. ruminicola	Sheep rumen	Monocultures	PMSF	Serine protease inhibitor	Low inhibition	(*35*)
				3,4-Dichloroisocoumarin		Moderate inhibition	
				Iodoacetate	Glyceraldehyde-3-phosphate dehydrogenase inhibitor	No inhibition	
				L-Trans-epoxysuccinyl-leucylamido(4-guanidino) butane (E-64)	Cysteine proteases inhibitor	No inhibition	
				Pepstatin	Aspartyl proteases inhibitor	Low inhibition	
				1,10-Phenanthroline	Metal chelating agent	No inhibition	
				Bestatin	Aminopeptidase B and leucine aminopeptidase inhibitor	Moderate inhibition	
				Diprotin A	hDPP-4 inhibitor	Complete inhibition	
				EDTA	Chelating agent	Low inhibition	
	P. merdae (DSM19495)	Type strain	Monocultures: purified mDPP-4	Sitagliptin and linagliptin	hDPP-4 inhibitor	Low inhibition	(*34*)
				Saxagliptin and vildagliptin		Moderate inhibition	

(*Continued*)

(*Continued*)

Peptidase type	Bacterial species	Source	Study Type	Inhibitor/s	Inhibitor/s class	Enzymatic activity	Reference
PepX	*L. lactis* subsp. *cremoris*	–	Monocultures: purified PepX	Valine-pyrrolidide	hDPP-4 inhibitors	Moderate inhibition	(*33*)
				Diprotin A		No inhibition	
				Diprotin B		No inhibition	
	Lactobacillus reuteri (100-23)	Type strain	Monocultures	Vildagliptin	hDPP-4 inhibitor	No inhibition and no effect of growth	(*134*)
	S. mutans UA159 (ATCC 700610)	Type strain	Monocultures	Saxagliptin, vildagliptin, and sitagliptin	hDPP-4 inhibitors	Inhibition of biofilm formation	(*193*)

reports on hDPP-4i impact on mDPP-4 activity. Last, an in vitro study showed that *Lactobacillus* isolates from human infant fecal samples, including *Lactobacillus plantarum*, *Lactobacillus fermentum*, and *Lactobacillus acidophilus*, heat-killed suspensions increased overall fluorometric DPP-4 activity (*137*). This body of research illustrates that hDPP-4is have variable to limited effects on mDPP-4; this variability could be attributed to the species or strains carrying the enzymatic activity within the host microbiome, suggesting the need for the development of mDPP-4 screening assays and personalized microbiome-targeted mDPP-4 inhibitors (mDPP-4is).

Microbially derived DPP-4is

As the prevalence of metabolic disorders like T2DM and obesity and neurological conditions like AD, PD, and MCI increase worldwide, the interest in exploring mDPP-4i has grown, especially given the reported side effects of some synthetic hDPP-4i, which include infections, pancreatitis, musculoskeletal disorders, reduced fertility, headaches, and joint pain (*24*, *138*).

Before the development of gliptins in 2006, the first DPP-4is, identified in 1984, were diprotin A (Ile-Pro-Ile) and diprotin B (Val-Pro-Leu), which were isolated from bacteria (*139*). Early studies showed that microbial metabolites such as diprotin A from *Bacillus cereus* BMF673-RF1 and sulfostin S from *Streptomyces* sp. MK251-43F3 had mDPP-4i activity (*140*, *141*). Subsequent studies demonstrated the presence of mDPP-4i in *Lactobacillus* (Table 3) (*142*–*144*), highlighting their potential role in modulating gut peptide metabolism. An in vitro study examining the interaction between Caco-2 cells and *L. plantarum* YE4 showed that mRNA expression of hDPP-4 in the cell line was significantly suppressed when treated with *L. plantarum* YE4 cell-free extracts (CFEs) by altering the tumor necrosis factor and mitogen-activated protein kinase (MAPK) signaling pathways. The main potential active components identified in the CFE were adenine, acetylcholine, and L-phenylalanine (*145*). Likewise, administration of cell-free supernatants and CFE from *L. acidophilus* KLDS1.0901 decreased fasting blood glucose levels, glycosylated hemoglobin and insulin in serum, and increased the level of GLP-1 in serum compared with diabetic untreated mice (*143*). In vitro studies of the impact of probiotic strains in combination with prebiotics on GLP-1 secretion revealed that *Lactobacillus paracasei* LC-37 and isomaltooligosaccharide and *Bifidobacterium animalis* MN-Gup and GOS enhanced GLP-1 secretion in NCI-H716 cells (*146*). Considering the evidence presented above, investigating strains with DPP-4 inhibitory effects may offer a potential avenue for addressing metabolic and neurological disorders regulated by DPP-4 substrates, suggesting an area for future research.

DPP-4 SUBSTRATES AND THEIR ASSOCIATION WITH THE GUT MICROBIOTA, METABOLIC DISORDERS, AND BRAIN HEALTH

hDPP-4 has been shown to regulate the functionality of more than 40 potential bioactive substrates, which are implicated in gut homeostasis, inflammation, and metabolic balance and are thought to influence cognition and behavior (*5*, *6*). Human and microbial DPP-4 substrates encompass a wide range of peptides, including incretin hormones from the glucagon family peptides such as GLP-1, GLP-2, GIP, and pituitary adenylate cyclase-activating polypeptide; bioactive pancreatic polypeptides (PPs) such as NPY and PYY; chemokines such as stromal-derived factor-1α (also known as CXCL-12); and neuropeptides such as SP, brain natriuretic peptide, and gastrin-releasing polypeptide (Table 4) (*25*, *58*). When human or microbial DPP-4 truncates a substrate, it can modulate receptor selectivity or completely inactivate the peptide, resulting in variable physiological responses. For instance, when active GLP-$1_{9\text{-}37}$ and GLP-$1_{9\text{-}36NH2}$ are cleaved to their inactive metabolites, GLP-$1_{7\text{-}37}$ and GLP-$1_{7\text{-}36NH2}$, respectively, they are incapable of binding to the GLP-1R and increase postprandial insulin production (*147*). However, cleaved GLP-1 is involved in other non-insulinotropic functions (*148*, *149*). Generally, after being truncated by DPP-4, most substrates that lack the X-proline N-terminal dipeptide are rapidly degraded by other peptidases. This phenomenon has been observed in SP and GLP-1, further degraded by aminopeptidase N and neprilysin (*150*, *151*).

It has not been confirmed whether DPP-4 substrates are present in the gut lumen, as some studies argue that they are mainly found in circulation or on the intestinal capillary endothelium (*152*). Olivares *et al.* (*6*) proposed that DPP-4 substrates are not typically found in the gut lumen. Still, a possible translocation of mDPP-4 and PepX to host tissues is possible (Fig. 3). The group led by Olivares, Hernández-Calderón, Cárdenas-Brito, Liébana-García, Sanz and Benítez-Páez (*34*) recently provided experimental evidence supporting the potential translocation of mDPP-4 from the gut lumen to host tissues. Their study used *E. coli* (BL21-DE) expressing the full-length *P. merdae* DPP-4 (pmDPP-4) to investigate the localization and potential movement of pmDPP-4.

Table 3. Microbial strains with reported DPP-4i activity. ND, not determined.

Phylum	Strain	Source	Type of study	Active molecule	Reference
Bacillota (formerly *Firmicutes*)	*B. cereus* BMF673-RF1	Type strain	In vitro	Diprotin A	(*140*, *141*)
	L. plantarum YE4	Chinese Qula, yogurt, and preserved pickles	In vitro	Adenine, acetylcholine, and L-phenylalanine	(*145*)
	Lactobacillus rhamnosus KLDS1.0205/ KLDS1.0911/KLDS1.0912	Traditional fermented products	In vitro/in vivo	ND	(*143*)
	L. rhamnosus GG (ATCC 53103)	Type strain	In vitro/in vivo	ND	
	L. plantarum KLDS1.0317/1.0318/1.0344/1.0386	Traditional fermented products	In vitro	ND	
	L. acidophilus KLDS1.1003/1.0901/1.0902	Traditional fermented products	In vitro	ND	
	L. paracasei KLDS1.0351	Traditional fermented products	In vitro	ND	
	Lactobacillus helveticus KLDS1.0903	Traditional fermented products	In vitro	ND	
	Lactobacillus bulgaricus KLDS1.0207	Traditional fermented products	In vitro	ND	
	L. plantarum (KC491380/KF678450/	Fecal isolate	In vitro	ND	(*137*)
	L. fermentum KC866340	Fecal isolate	In vitro	ND	
	Bacillus amyloliquefaciens	Commercial strain	In vitro	γ-Glutamyl dipeptides	(*201*)
	L. rhamnosus MW049146	Infant feces	In vitro	ND	(*202*)
	Pediococcus acidilactici	Fermented soybean (Bekang)	In vitro	ND	
	Levilactobacillus brevis MW051598	Infant feces	In vitro	ND	
	Lactiplantibacillus plantarum MW055659	Infant feces	In vitro	ND	
	Lactiplantibacillus plantarum MW055704	Beetroot	In vitro	ND	
Bacteroidota	*Chryseobacterium* sp. kr6	Poultry processing waste	In vitro	Keratin hydrolysate	(*203*)
	Bacteroides acidifaciens (JCM10556)	Type strain	In vitro/in vivo	ND	(*204*)
Actinomycetota	*Streptomyces* sp. MK251-43F3	Soil	In vitro	Sulfostin S	(*140*, *141*, *205*)
	Bifidobacterium bifidum NCIMB 702715	Type strain	In vitro	ND	(*137*)
	Bifidobacterium longum subspecies *longum* (CP002286)	Centenarian's feces	In vitro	ND	(*205*)
	Bifidobacterium animalis subspecies *lactis* (GU116483.1)	Commercial probiotic	In vitro	ND	
	Bifidobacterium adolescentis (KP256212/ KP256213/KP256215/KP256214)	Infant feces	In vitro	ND	
	B. longum (KP256208/KP256209/ KP256207)	Infant feces	In vitro	ND	
	Bifidobacterium breve (KP256218/ KP256219)	Infant feces	In vitro	ND	
	Bifidobacterium catenulatum (KP256216/ KP256217/KP256211)	Infant feces	In vitro	ND	
	B. bifidum (KP256210)	Infant feces	In vitro	ND	
Fungi	*Aspergillus oryzae* strain AO-1	Fermented food	In vitro	Tetrahydroxyisoquinoline derivative	(*207*)

Immunogold staining combined with transmission electron microscopy (TEM) revealed that pmDPP-4 localized both in the inner membrane and within the extracellular polysaccharide capsule, suggesting that it may be accessible for interactions beyond the bacterial cell. Other studies showed that this could be achieved by BEVs, such as those generated by *B. thetaiotaomicron*, as they encompass multiple enzymatic activities, including m-DPP-4 (*67*). Using labeled *B. thetaiotaomicron*–generated BEVs in mice and intestinal epithelial organoids, Jones, Booth, Fonseca, Parker, Cross, Miquel-Clopés, Hautefort, Mayer, Wileman and Stentz (*153*) elucidated several pathways for BEVs to access systemic organs. One identified route involved caveolin-mediated endocytosis, leading to the transport of BEVs to the endoplasmic reticulum and Golgi network. Additionally, they revealed that BEVs can travel through endolysosomal vesicles positioned near the perinuclear membrane. Moreover, BEVs can traverse paracellularly between epithelial cells, migrating through tight junctions (*153*). In alignment with these findings, gut microbiome-derived BEVs have been detected in the plasma of healthy individuals and patients with IBD (*154*). This hypothesis is further supported by mammalian DDP-4 KO in conventional mice having ~10% DDP-4 activity detected in serum compared to WT mice (*61*) and by a recent study that showed that compromising the barrier function via HFD or DSS was essential to detect mDPP-4 in mice serum (*3*). Furthermore, Olivares *et al.* (*34*) demonstrated that administering *E. coli*–expressing pmDPP-4 to mice, combined with increased gut

Table 4. Example of DPP-4 substrates: Roles in brain health, neurodegeneration, and microbiome involvement. BBB, blood-brain barrier; BNP, brain natriuretic peptide; CNS, central nervous system; CSF, cerebrospinal fluid; GI, gastrointestinal; GIPR, GIP receptor; GLP-1R, GLP-1 receptor; GLP-2R, GLP-2 receptor; GRP, gastrin-releasing polypeptide; GRPR, GRP receptor; GSK-3β, glycogen synthase kinase 3β; HMGB1, HMG box 1; Kv, voltage-gated potassium; LPS, lipopolysaccharide; NAD+H, nicotinamide adenine dinucleotide (NAD) + hydrogen (H); OXM, oxyntomodulin; PACAP, pituitary adenylate cyclase-activating polypeptide; RAGE, receptor for advanced glycation endproducts; SCFAs, short-chain fatty acids; SDF-1, stromal-derived factor-1; TLR, Toll-like receptor; 5-HT, 5-hydroxytryptamine receptor.

Peptide family	Peptide	Biological structure	Receptor	Receptor/ substrate locations	Cleaved peptide	DPP-4 effect	Role in brain health	Microbiome involvement	Microbial metabolites involvement	Reference
Incretin hormones	GLP-1 (GLP-1_{7-37} and GLP-$1_{7-36NH2}$)	30–Amino acid peptide hormone	GLP-1R	Endocrine pancreas, central/peripheral nervous systems, GI tract (L cell of distal intestine), cardiovascular system, kidneys, and lungs	GLP-1_{9-37} and GLP-$1_{9-36NH2}$	Metabolically inactive GLP-1, with circulating half-life of 1 to 2 min	Protects against excitotoxic cell death and oxidative injury	Decrease in *Bacillota* and *Bacteroidota* and an increase in *Pseudomonadota* related to higher levels of GLP-1		(*25*, *208–211*)
							Counters effects of amyloid-β (Aβ) 1-42, linked to AD		Acetate, butyrate, and propionate	
							Overexpression of receptors improves spatial learning in mice.		Indole	
							Induces neurite outgrowth		LPS	
							Promotes brain progenitor cell proliferation			
							Neuronal tissue regeneration			
							Neuroprotective effects in AD and PD animal models			
	GIP	42–Amino acid peptide hormone	GIPR	Small intestine (K cell of proximal intestine), adipose tissue, adrenal cortex, heart, lung, testis, bone, pituitary, and brain	GIP_{3-42}	Inactivation, with circulating half-life of 7 min	Neuroprotective effects in AD and PD animal models	An increase in the *Bacillota*/*Bacteroidota* ratio is potentially related to higher levels of GIP.		(*25*, *172*, *175*, *208*, *209*, *212–217*)
							Decreases Aβ plaque formation in the cortex			
							Stimulates neuronal proliferation			
							Protects synapse function and numbers		SCFAs, especially propionate	
							Reduces chronic neuroinflammation response	An increase in *Akkermansia* is negatively associated with GIP levels.	Indole propionate	
							Reduces oxidative stress			
							Modulates neurotransmitter release			
							Influences long-term potentiation formation for learning and memory protection			
	GLP-2	33–Amino acid peptide	GLP-2R	Stomach, small and large intestine (in enteric nerves and enteroendocrine cells), brain, and lungs	$GLP-2_{3-33}$	Inactivation, with circulating half-life of 7 min	Restores neurogenesis in AD mouse model	An increase in *Bifidobacterium* and supplementation with probiotic *Bifidobacterium animalis* are related to the higher levels of GLP-2.	SCFAs	(*218–222*)
							Improves spatial learning memory			
							Alleviates oxidative stress			
							Enhances insulin sensitivity via PI3K signaling			
							Inhibits apoptosis	Intake of *Lactobacillus reuteri* increases the release of GLP-2.		
							Reduces oxidative stress			
							Decreases neuroinflammation			

(*Continued*)

(*Continued*)

Peptide family	Peptide	Biological structure	Receptor	Receptor/ substrate locations	Cleaved peptide	DPP-4 effect	Role in brain health	Microbiome involvement	Microbial metabolites involvement	Reference
	OXM	37–Amino acid peptide	GLP-1R and the glucagon receptor	Liver, brain, pancreas, kidney, adipose tissue, and gut (especially L cells).	OXM_{3-39}	Inactivation, with circulating half-life of 11 to 13 min	An effective regulator of appetite Enhances synaptic plasticity Reduces Aβ levels and restores the PI3K/AKT/GSK3β cell signaling pathway in the hippocampus. Potential treatment of AD	*Bifidobacterium longum* expresses OXM.	No evidence	(*223–226*)
Pancreatic polypeptides	NPY (NPY_{1-36})	36–Amino acid neuropeptide	Y receptors (Y1, Y2, Y4, Y5, and Y6)	Brain (hypothalamus, hippocampus, neocortex, and thalamus), adipose tissue, kidney, adrenal gland, heart, and placenta, GI tract (L cell of distal intestine "PYY")	NPY_{3-36}	Modified binding preference and inactivation at Y1-receptor, with circulating half-life of 6 to 9 min	Promotes autophagy Reduces excitotoxicity Inhibits neuroinflammation	Increase in *Lactobacillus* spp. and *Clostridium cocleatum* and decrease in *Burkholderiales* correlate with increasing in NPY.	SCFAs Tryptophan	(*227–229*)
	PYY (PYY_{1-36})	36–Amino acid neuropeptide			PYY_{3-36}		Involves in depressive and anxiety Affects behavior and cognition Involves in satiety regulation and energy homeostasis	Supplementation of probiotics (*Lactobacillus* and *Saccharomycetes* strains) increases the levels of PYY.	Tyrosine Glutamine	(*208*, *228*, *230–234*)
Tachykinin peptide family	SP (SP_{1-11})	11–Amino acid neuropeptide	Neurokinin 1 receptor	Lymphocytes, neural tissue, brain, and GI (in the enteric efferent neurons)	SP_{3-11} and SP_{5-11}	Inactivation	Major mediator of neurogenic inflammation CNS function neurotransmitter Affects behavior and neurochemical responses to psychological and somatic stress Neuroprotective against AD by promoting non-amyloidogenic APP processing and inhibiting Aβ deposition via Kv channel regulation	Gut microbiota (e.g., *Clostridium*) promote synthesis of 5-HT and up-regulates the release of SP.	Tryptophan	(*25*, *30*, *235–238*)
Chemokines	RANTES (CCL-5)	68–Amino acid peptide	G protein–coupled receptor family (namely, CCR1, CCR3, CCR4, and CCR5)	Lymphoid organs, surface of T cells and macrophages, brain, endothelial cells.	$RANTES_{3-68}$	Inactivation at CCR1 and CCR3 receptors, but not at CCR5	Promotes leukocyte infiltration to sites of inflammation Pro-inflammatory markers in generalized anxiety disorder and personality disorders Modulates calcium currents in central neurons Slows the rate of apoptotic death of hippocampal neurons Stimulates the expression of genes involved in neuronal survival, neurite outgrowth, and synaptogenesis	Increase in *Enterococcus*, *Streptococcus*, and *Vagococcus* is associated with higher production of CCL-5. Supplementation with anti-inflammatory *Bifidobacterium* strains decreased the expression of CCL-5.	Propionate	(*239–244*)

(*Continued*)

(*Continued*)

Peptide family	Peptide	Biological structure	Receptor	Receptor/ substrate locations	Cleaved peptide	DPP-4 effect	Role in brain health	Microbiome involvement	Microbial metabolites involvement	Reference
	SDF-1 (CXCL-12)	SDF-1α (68 amino acids) SDF-1β (72 amino acids)	CXCR4/ CXCR7	Liver, lung, brain, kidney, heart, colon, lymph nodes, skin and bone marrow, and endothelial cells	SDF-1$\alpha_{3\text{-}67}$ and SDF-1$\beta_{3\text{-}72}$	Changes in activity, inactivation at CXCR4 receptor	Promotes neurite outgrowth	*B. ovatus* and *B. xylanisolvens* are potentially correlated with the levels of SDF-1	Butyrate	(*25*, *245–253*)
							Enhances neurogenesis			
							Facilitates migration of neural progenitor cells to brain lesion sites	Lower abundance of *Firmicutes* and higher abundance of *Ruminococcaceae* is associated with higher levels of SDF-1β.		
							Reduces Aβ accumulation			
							Involves in cognitive function			
Damage-associated molecular pattern	HMGB1 (or amphoterin	215–Amino acid peptide	RAGE, TLR9, and TLR5	Heart, brain, and lymphoid organs	HMGB1$_{17\text{-}215}$	Non effectively inactivation	Increases vascular permeability and promotes BBB decomposition	Gram-negative bacteria stimulate the release of HMGB1.	LPS	(*254–257*)
							Promotes brain remodeling in the recovery phase of ischemic stroke			
							Promotes recovery of neurological function			
Natriuretic peptides	BNP	32–Amino acid peptide	Natriuretic peptide receptor-A	Heart, brain, and kidney	BNP$_{3\text{-}32}$ and BNP$_{5\text{-}32}$	Uncleared inhibition	Higher plasma levels of BNP are associated with lower CSF levels of Aβ42 and total tau/Aβ42 ratios	No evidence	No evidence	(*25*, *258*, *259*)
Bombesin-like peptide	GRP	27–Amino acid neuropeptides	GRP receptor	Brain, gut, pancreas, prostate, and lung	GRP$_{3\text{-}27}$ and GRP$_{5\text{-}27}$	Uncleared inhibition	Neuroprotection by indirectly stimulating GLP-1 secretion	No evidence	LPS	(*25*, *225*, *260*, *261*)
							GRPR signaling might integrate the processing of stress and fear with synaptic plasticity and memory.			
							Prevent Aβ-induced cognitive impairment			
Vasoactive intestinal peptide	PACAP	38–Amino acid neuropeptides	G protein–coupled PAC1 receptor and VPAC1 and VPAC2	Brain, endocrine organs, heart, gut, and lymphoid organs	PACAP$_{3\text{-}38}$	Inactivation	Against Aβ-induced neuronal toxicity and increase in levels of the Aβ-degrading enzyme neprilysin	*Bacteroides/Prevotella* spp. levels are associated with PACAP expression.	SCFAs	(*25*, *262–264*)
							Antiapoptotic properties and are involved in learning and memory processes	Vasoactive intestinal peptide deficiency is associated with altered gut microbiota communities.	NAD(P)H	
									LPS	

permeability induced by lipopolysaccharide (LPS) injection, resulted in a significant rise in DPP-4 activity in the mice portal vein.

Conversely, another potential route was shown by a recent study using an ex vivo gut model that provided evidence of the apical secretion of PYY and GLP-1 at the gut lumen in unstimulated and enteroendocrine stimulatory conditions (*155*). Additionally, Stevens *et al.* (*156*) demonstrated the luminal secretion of PYY and GLP-1 from human colon monolayers in response to the apical treatment with rebaudioside A or casein.

Glucagon-like peptide-1

GLP-1 is a 30–amino acid incretin hormone derived from the proglucagon gene, first described in the 1980s (*157*, *158*). The half-life of GLP-1 is notably short, ~1 to 2 min, as it is rapidly inactivated by

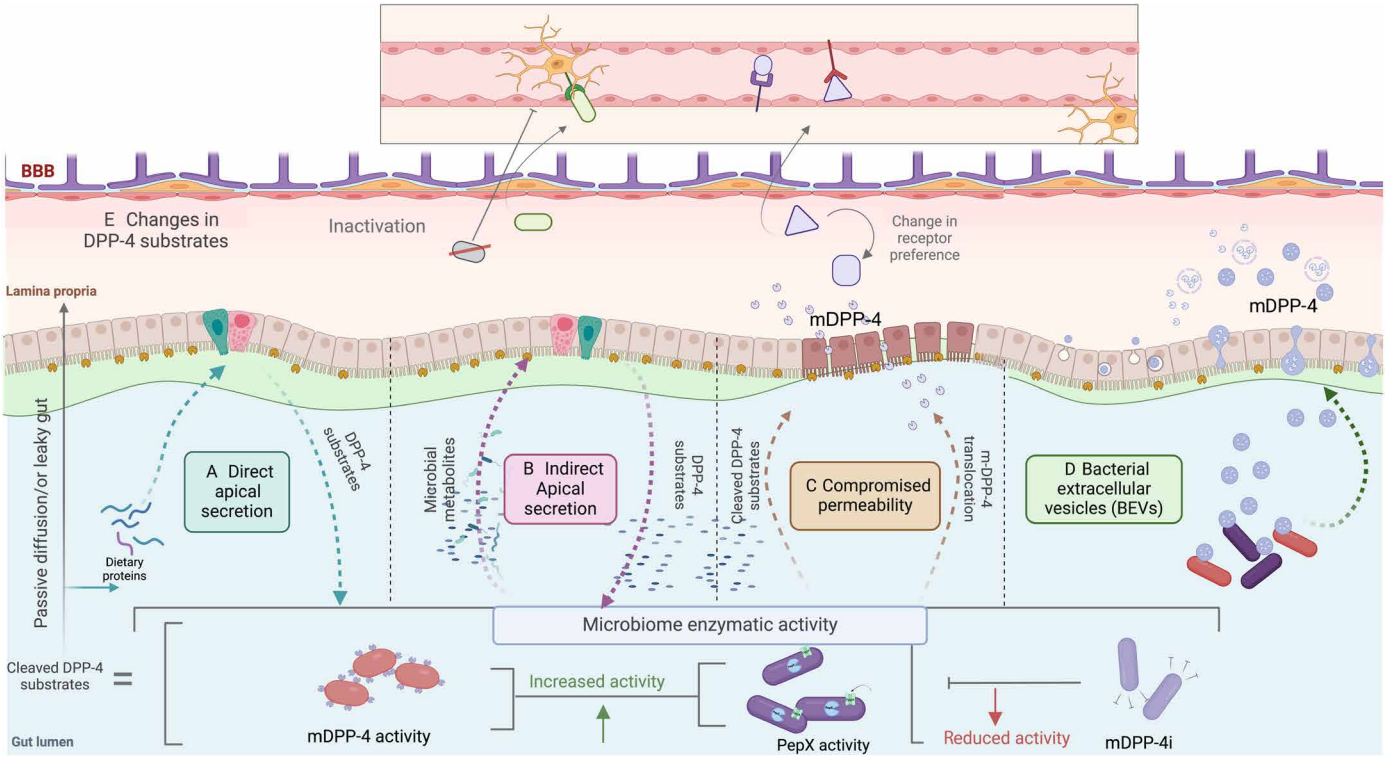

Fig. 3. Potential microbial DPP-4 and PepX pathways. (**A**) Direct apical secretion pathway; dietary proteins stimulate the production of DPP-4 substrates from L and K cells into both circulation and luminal spaces of the gut; these substrates are then cleaved by mDPP-4 and PepX activities. (**B**) Indirect apical secretion pathway; microbial metabolites (e.g., tryptophan metabolites) and the production of DPP-4 substrates from L cells both circulation and luminal spaces of the gut; these substrates are then cleaved by mDPP-4 and PepX activities. (**C**) Compromised permeability pathway; cleaved DPP-4 substrates by mDPP-4 and PepX activities cross the epithelial barrier into the blood-brain barrier (BBB), or mDPP-4 enzyme is translocated into the BBB where it acts on DPP-4 substrates there. (**D**) Bacterial extracellular vesicles (BEVs) pathway that contain mDPP-4 enzyme and can pass the epithelial barrier by caveolin-mediated endocytosis, endolysosomal vesicles, and transmigrating through tight junctions. (**E**) Cleaved DPP-4 substrates cross the epithelial barrier by passive diffusion or by leaky gut, and either the cleaved DPP-4 substrates are converted into metabolically inactive substrate and cannot bind to its receptor or the cleaved substrates change receptor preference. This figure was made using BioRender.

DPP-4 (*46*). GLP-1 has been extensively used as a drug target due to its diverse physiological roles, which include glucose-lowering effects by promoting insulin secretion and suppressing glucagon release (*159*, *160*). GLP-1 is synthesized and secreted as two bioactive forms, GLP-$1_{7\text{-}36}$ and GLP-$1_{7\text{-}37}$, which result from the alternative posttranslational processing of the proglucagon gene (*152*). Both isoforms are equipotent and bind with equal affinity to the GLP-1R, a G protein–coupled receptor found on numerous cell types, including pancreatic β cells and neurons (*161*). GLP-1R is expressed in various peripheral organs and brain regions, including areas essential for cognitive and motor functions and metabolic regulation, as well as in microglial cells involved in neuroinflammation regulation, with both GLP-1 and GLP-1R reported to being down-regulated in AD brains (*99*, *162*, *163*). GLP-1 is considered the most researched DPP-4 substrate, with substantial laboratory and clinical evidence supporting its potential role in treating neurodegenerative diseases by three main proposed pathways: the GLP-1R/phosphatidylinositol 3-kinase (PI3K)/Akt, GLP-1R/PI3K/MAPK, and GLP-1R/cyclic adenosine 3′,5′-monophosphate (cAMP)/cAMP-dependent protein kinase [reviewed in (*25*)]. Recently, FDA-approved GLP-1 receptor agonists (GLP-1 RAs) medications, including liraglutide, dulaglutide, and exenatide, have been associated with a reduced risk of anxiety and depression (*164*). Recent clinical trials on GLP-1 RAs have also shown promising results in lowering the prevalence of neurological complications of T2DM, such as stroke, cognitive impairment, and peripheral neuropathy [reviewed in (*165*)].

Injecting mice intravenously with mDPP-4 cloned and purified from *P. gingivalis*, *P. intermedia*, and *T. forsythia* led to decreased plasma active GLP-1 and insulin levels following oral glucose administration, illustrating that mDPP-4 can influence GLP-1 in a manner akin to mammalian DPP-4 (*62*). Further, the gut microbiota has been shown to modulate GLP-1 production through microbial metabolites such as short-chain fatty acids (SCFAs) by activating G protein–coupled FFAR2-3 in L cells by elevating intracellular Ca^{2+} and tryptophan-derived metabolites like indole, which can stimulate GLP-1 release from intestinal L cells by inhibiting voltage-gated K^{+} channels and rising Ca^{2+} influx (*135*, *155*, *166*). Additionally, LPS from Gram-negative gut bacteria can bind to Toll-like receptor 4 in L cells and increase GLP-1 production (*167*, *168*). A randomized controlled trial assessed the effects of a 3-month almond-based low-carbohydrate diet on 45 patients with T2DM. The dietary intervention notably increased the relative abundance of SCFA-producing bacteria, namely, *Roseburia*, *Ruminococcus*, and *Eubacterium*. These changes in relative abundance were linked to higher GLP-1 concentrations, correlating with improved depression symptoms (*169*). Another example of the SCFA pathway is the use of phloretin, an inhibitor of glucose

transporter type 2, which has been observed to suppress maltose/miglitol-induced GLP-1 secretion in mice by inhibiting SCFAs production by the microbiome (*170*, *171*).

Gastric inhibitory polypeptide

GIP was discovered as an incretin hormone over a decade before GLP-1 (*157*). GIP is made of 42 amino acids and is mainly secreted by K cells in the duodenum and proximal jejunum (*172*). Like GLP-1, the GIP receptor (GIPR) is present in various tissues, including the small intestine, adipose tissue, adrenal cortex, heart, lung, testis, bone, pituitary gland, and brain (*25*). GIP and protease-resistant GIP analogs exhibit neuroprotective cognitive effects in animal models of AD and PD (*173*), and, recently, GLP-1R/GIPR dual agonists have been shown to improve cognitive functions, memory retention, and motor function (*174*). Although initially developed for managing T2DM, these findings have broadened the potential therapeutic applications of DPP-4 substrate agonists.

Microbiome changes induced by sleeve gastrectomy have been shown to contribute to reducing GIP signaling and confer resistance against diet-induced obesity (DIO) and nonalcoholic fatty liver disease (NAFLD). *Bacteroides* and *Akkermansia* were found to be negatively associated with GIP levels and indole and propionate levels after bariatric surgery (*175*). Mice colonized with stools from patients after bariatric surgery were more resistant to DIO and NAFLD development, and this resistance was associated with a reduction in GIP levels. These findings indicated that the positive effect of bariatric surgery on NAFLD was partly due to microbiome changes. Male Wistar rats fed dietary fiber oligofructose for 30 days had significantly lower serum insulin levels. Additionally, serum and jejunal GIP concentrations and cecal GLP-1 levels were significantly higher in the serum of oligofructose-fed rats (*176*). GIP has been less studied compared to GLP-1 in the context of the gut microbiome. Further research could reveal novel insights and potential therapeutic applications related to GIP and its interactions with the gut microbiota.

NPY and PYY

NPY and PYY are highly conserved peptides that belong to the PP family, first identified in the 1980s (*177*). NPY is a 36–amino acid neuropeptide expressed in various central nervous system cell types, including enteric and sympathetic neurons. PYY is a 36–amino acid peptide exclusively produced by enteroendocrine cells in the gut (*6*, *28*). Both peptides share a similar structure, which includes a feature known as the PP-fold, a hairpin-like conformation stabilized by a conserved disulfide bond formed between two cysteine residues that have been shown to be important for their interaction with specific receptors (*178*). The involvement of the NPY family of peptides in gut-brain communication is supported by the presence of five NPY receptor types, namely, Y1, Y2, Y4, Y5, and Y6 (a human pseudogene), found along the gut-brain signaling pathways (*179*).

NPY and PYY are crucial in regulating energy balance, feeding behavior, and stress response and have been implicated in cognitive functions. In particular, NPY has been shown to have neuroprotective and anxiolytic effects and has been associated with learning and memory processes (*25*, *180*, *181*). In patients with AD, a reduction of NPY and decreased binding of PYY and NPY to their receptors have been shown in plasma, in cerebrospinal fluid, and in the temporal cortex and hippocampus (*182*–*184*). SCFAs, particularly butyrate, can induce PYY secretion. Upon secretion, a portion of PYY is enzymatically cleaved by DPP-4 to $PYY_{3\text{-}36}$, which is the predominant circulating form of PYY, leading to increased gastric pressure via stimulation of Y2 receptors, eventually resulting in slower gastrointestinal movement and the secretion of electrolytes and water through both neural and nonneural pathways (*28*, *185*). HFD mice exhibited depression-like behavior, decreased levels of NPY in the hypothalamus and hippocampus, and increased plasma NPY and DPP-4 activity. The depression-like behavior was induced by prolonged HFD and was associated with distinct alterations in the intestinal microbiome. The HFD-induced behavioral disturbance was not revisable by DPP-4i or antidepressant treatment, suggesting that these changes may be challenging to reverse (*186*). Recent evidence demonstrated the potential role of mDPP-4 in modulating host metabolic functions, as pmDPP-4 in both in vitro and in vivo assays showed its ability to cleave and inactivate GLP-1, GIP, PYY, and NPY peptides with efficiency comparable to that of hDPP-4 (*34*).

S15 PEPTIDASES IN THE MICROBIOME ENZYMATIC LANDSCAPE

The family of S15 peptidase enzymes, specifically PepX, cleaves the same peptide bonds as DPP-4 (Fig. 4) (*6*). PepX has been extensively characterized in LAB, especially in dairy fermentations (*33*). The structural comparison between *Lactococcus lactis* PepX and hDPP-4 revealed that, despite their significantly different overall folds, the most crucial residues for hDPP-4 activity are conserved in both enzymes, occupying the same positions and orientations. Likewise, PepX from *L. lactis* has been reported to have comparable sensitivity to DPP-4i valine-pyrrolidine (*33*). The main difference between DPP-4 and PepX is that the latter is usually associated with an oligopeptide transporter encoded by the *opp* operon, which transports the substrate into the cell (*187*). PepX was initially considered to function exclusively as an intracellular enzyme; however, recent findings demonstrated that it also has extracellular peptidase activity. For instance, although located in the cytoplasm, PepX from *Streptococcus thermophilus* contributes to the extracellular degradation of proline-rich peptides without inducing cell lysis or releasing intracellular peptidases due to a particular membrane orientation (*188*).

PepX has been reported in species of the genera *Lactobacillus*, *Lactococcus*, and *Streptococcus* (*18*, *189*). An in vitro study showed that monocultures of *L. acidophilus*, *L. fermentum*, *Lactobacillus johnsonii*, and *L. plantarum* carrying PepX resulted in enzymatic activity equivalent to DPP-4 when incubated with Gly-Pro-AMC (*137*). Conversely, one study reported that *L. plantarum* YE4 suppressed DPP-4 activity in Caco-2 cells (*145*), hinting at specific regulatory mechanisms that could help maintain an equilibrium between mDPP-4 and PepX activities in the gut (*137*). In this context, a recent study evaluated the potential use of probiotic-derived PepX to break down gluten peptides in wheat, which has been implicated in the regulation of autoimmune responses, as seen in conditions like celiac disease (*6*, *190*). On the other hand, PepX has been involved in the pathogenicity of *Streptococcus gordonii*, *Streptococcus agalactiae*, *Streptococcus pneumoniae*, and *Streptococcus pyogenes* (*190*–*192*). DPP-4i inhibits *Streptococcus mutans* biofilm formation, indicating potential use as antibiofilm therapy for oral health benefits (*193*). Further, an in vitro inhibition assay revealed that DPP-4i valine pyrrolidide effectively inhibited PepX from *L. lactis*. However, the DPP-4i tripeptides, diprotin A and diprotin B, showed limited inhibitory effects (*33*).

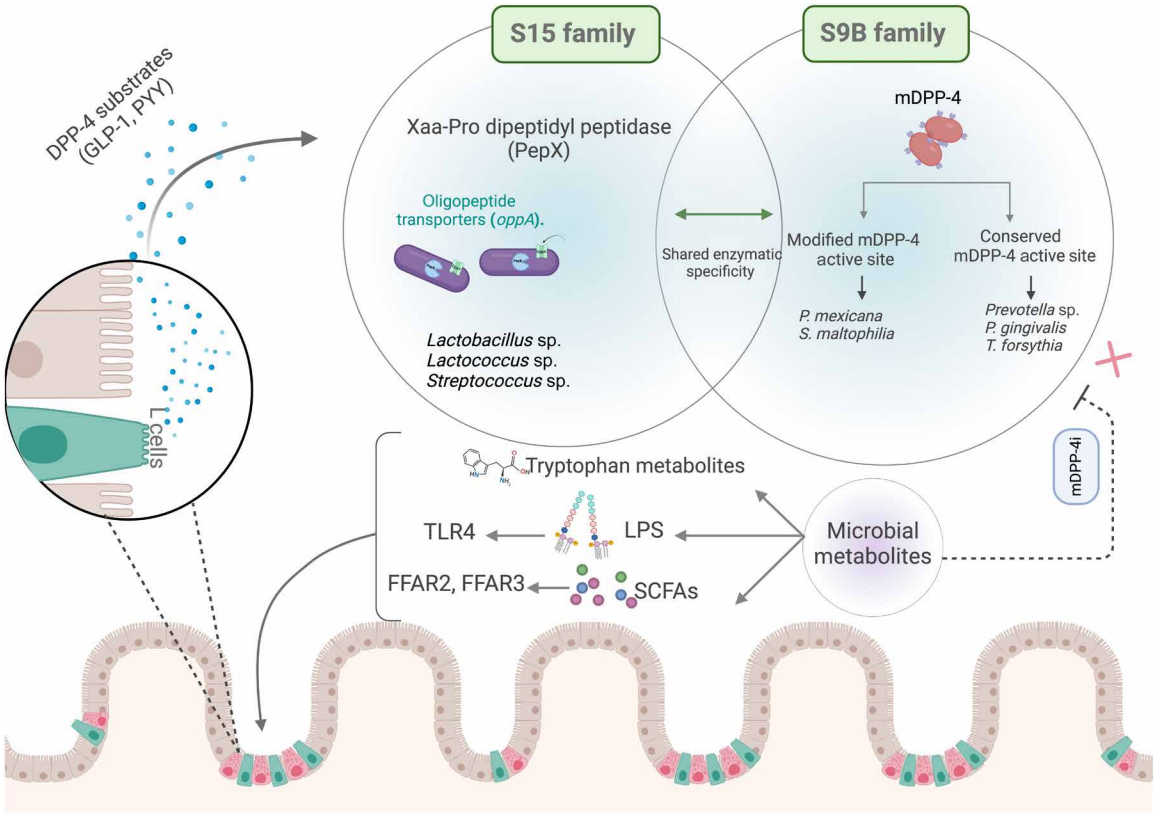

Fig. 4. Potential equilibrium dynamics of microbial DPP-4 and PepX enzymes. S9B and S15 family peptides are shown with their associated genera. The central Venn diagram highlights shared substrate specificity between these families. Below, the circle delineates microbial metabolites and outcomes: On the right, mDPP-4i metabolites with the ability to inhibit the activity of mDPP-4. On the left are three pathways where the microbiome can stimulate the production of DPP-4 substrates. This figure was made using BioRender.

DISCUSSION

The symbiotic relationship between microorganisms and their host is a well-established phenomenon, evidenced by the state of homeostasis that contributes to overall health. A recent study in *Drosophila melanogaster* showed that the microbiome composition drives rapid host genomic adaptation and that specific manipulations (e.g., varying the relative proportion of acetic acid bacteria versus LAB) were sufficient to cause genomic divergence of host populations over only five generations (*194*). However, the bacterial genetic components affecting this balance have been mostly researched in infectious diseases (*195*, *196*).

There is a growing interest in the impact of proteases and peptidases released by commensal bacteria on gastrointestinal diseases (*197*). Specifically, it is now understood that HMIs play a substantial role in the development of disease and responses to interventions. Two bacterial families of peptidases, the S9B and S15 families, have been reported in gut and oral microbiomes. mDPP-4, from the S9B family of bacterial peptidases, has been described in species of *Prevotella* and *Bacteroides* and oral pathogens like *P. gingivalis* and *T. forsythia.* In contrast, the S15 family is widely distributed in LAB. Exploring a competing balance between these two families of peptidases and their bacterial hosts, which could be adjusted to maintain optimal health, is an intriguing possibility worth considering.

While hDPP-4 has been extensively researched and identified as a substantial contributor to various diseases, the contribution of mDPP-4 to these diseases remains largely underexplored, even when hDPP-4is, originally developed for the treatment of diabetes, have shown promising benefits for multiple health conditions due to their influence on a range of substrates associated with systemic diseases, including inflammatory, obesity-related, cardiovascular, pulmonary, and neurodegenerative conditions. These findings reinforce that DPP-4 can be a therapeutic target for multiple disorders. Moreover, research studies showed that DPP-4 activity is highly conserved across eukaryotic and prokaryotic organisms (*6*, *22*, *23*). From our perspective, gut microbial peptidases as a natural means of gaining a competitive advantage can lead to identifying specific microbial inhibitors for mDPP-4 and generating individualized strategies for microbiome modulation.

METHODS

In silico structural prediction and ligand docking approaches

Enzyme structures where no crystal structure was available were predicted using AlphaFold2 (*198*). When no crystal structure of the enzyme-ligand complex was available, docking was performed using AutoDock 4 (*199*). PyMOL (*200*) was used to superimpose all structures to *H. sapiens* structure.

REFERENCES AND NOTES

1. M. Azcarate-Peril, J. Roach, A. Marsh, W. D. Chey, W. J. Sandborn, A. J. Ritter, D. A. Savaiano, T. Klaenhammer, A double-blind, 377-subject randomized study identifies *Ruminococcus*, *Coprococcus*, *Christensenella*, and *Collinsella* as long-term potential key players in the modulation of the gut microbiome of lactose intolerant individuals by galacto-oligosaccharides. *Gut Microbes* **13**, 1957536 (2021).

2. W. Chey, W. Sandborn, A. J. Ritter, H. Foyt, M. A. Azcarate-Peril, D. A. Savaiano, Galacto-oligosaccharide RP-G28 improves multiple clinical outcomes in lactose-intolerant patients. *Nutrients* **12**, 1058 (2020).
3. K. Wang, Z. Zhang, J. Hang, J. Liu, F. Guo, Y. Ding, M. Li, Q. Nie, J. Lin, Y. Zhuo, L. Sun, X. Luo, Q. Zhong, C. Ye, C. Yun, Y. Zhang, J. Wang, R. Bao, Y. Pang, G. Wang, F. J. Gonzalez, X. Lei, J. Qiao, C. Jiang, Microbial-host-isozyme analyses reveal microbial DPP4 as a potential antidiabetic target. *Science* **381**, eadd5787 (2023).
4. N.-H. Kim, T. Yu, D. H. Lee, The nonglycemic actions of dipeptidyl peptidase-4 inhibitors. *Biomed. Res. Int.* **2014**, 368703 (2014).
5. A. Mortier, M. Gouwy, J. Van Damme, P. Proost, S. Struyf, CD26/dipeptidylpeptidase IV—chemokine interactions: Double-edged regulation of inflammation and tumor biology. *J. Leukoc. Biol.* **99**, 955–969 (2016).
6. M. Olivares, V. Schüppel, A. M. Hassan, M. Beaumont, A. M. Neyrinck, L. B. Bindels, A. Benítez-Páez, Y. Sanz, D. Haller, P. Holzer, N. M. Delzenne, The potential role of the dipeptidyl peptidase-4-like activity from the gut microbiota on the host health. *Front. Microbiol.* **9**, 1900 (2018).
7. N. D. Rawlings, A. J. Barrett, P. D. Thomas, X. Huang, A. Bateman, R. D. Finn, The MEROPS database of proteolytic enzymes, their substrates and inhibitors in 2017 and a comparison with peptidases in the PANTHER database. *Nucleic Acids Res.* **46**, D624–D632 (2018).
8. J. Potempa, R. N. Pike, Bacterial peptidases. *Contrib. Microbiol.* **12**, 132–180 (2005).
9. C. López-Otín, J. S. Bond, Proteases: Multifunctional enzymes in life and disease. *J. Biol. Chem.* **283**, 30433–30437 (2008).
10. L. P. Tripathi, R. Sowdhamini, Genome-wide survey of prokaryotic serine proteases: Analysis of distribution and domain architectures of five serine protease families in prokaryotes. *BMC Genomics* **9**, 549 (2008).
11. A. Jablaoui, A. Kriaa, H. Mkaouar, N. Akermi, S. Soussou, M. Wysocka, D. Wołoszyn, A. Amouri, A. Gargouri, E. Maguin, Fecal serine protease profiling in inflammatory bowel diseases. *Front. Cell. Infect. Microbiol.* **10**, 21 (2020).
12. S. Edogawa, A. L. Edwinson, S. A. Peters, L. L. Chikkamenahalli, W. Sundt, S. Graves, S. V. Gurunathan, M. Breen-Lyles, S. Johnson, R. Dyer, Serine proteases as luminal mediators of intestinal barrier dysfunction and symptom severity in IBS. *Gut* **69**, 62–73 (2020).
13. N. Rawlings, L. Polgar, A. Barrett, A new family of serine-type peptidases related to prolyl oligopeptidase. *Biochem. J.* **279**, 907–908 (1991).
14. V. K. Hopsu-Havu, G. G. Glenner, A new dipeptide naphthylamidase hydrolyzing glycyl-prolyl-β-naphthylamide. *Histochemie* **7**, 197–201 (1966).
15. C. Pethiyagoda, D. Welch, T. Fleming, Dipeptidyl peptidase IV (DPPIV) inhibits cellular invasion of melanoma cells. *Clin. Exp. Metastasis* **18**, 391–400 (2000).
16. J. J. Holst, C. F. Deacon, Inhibition of the activity of dipeptidyl-peptidase IV as a treatment for type 2 diabetes. *Diabetes* **47**, 1663–1670 (1998).
17. C. Deacon, S. Wamberg, P. Bie, T. Hughes, J. Holst, Preservation of active incretin hormones by inhibition of dipeptidyl peptidase IV suppresses meal-induced incretin secretion in dogs. *J. Endocrinol.* **172**, 355–362 (2002).
18. Y. Sanz, F. Toldrá, Purification and characterization of an X-prolyl-dipeptidyl peptidase from *Lactobacillus sakei*. *Appl. Environ. Microbiol.* **67**, 1815–1820 (2001).
19. E. Vernet, M. Ø. Pedersen, H. Thøgersen, A. C. Shaw, Engineering Xaa-Pro dipeptidyl aminopeptidase for specific cleavage of glucagon and glucagon-like peptide 1 from fusion proteins. *Protein Expr. Purif.* **170**, 105590 (2020).
20. G. Yüksel, J. Steele, DNA sequence analysis, expression, distribution, and physiological role of the Xaa-prolyldipeptidyl aminopeptidase gene from *Lactobacillus helveticus* CNRZ32. *Appl. Microbiol. Biotechnol.* **44**, 766–773 (1996).
21. B. Mayo, J. Kok, W. Bockelmann, A. Haandrikman, K. J. Leenhouts, G. Venema, Effect of X-prolyl dipeptidyl aminopeptidase deficiency on *Lactococcus lactis*. *Appl. Environ. Microbiol.* **59**, 2049–2055 (1993).
22. Y. Shibata, Y. Miwa, K. Hirai, S. Fujimura, Purification and partial characterization of a dipeptidyl peptidase from *Prevotella intermedia*. *Oral Microbiol. Immunol.* **18**, 196–198 (2003).
23. N. D. Walker, N. R. McEwan, R. J. Wallace, Cloning and functional expression of dipeptidyl peptidase IV from the ruminal bacterium *Prevotella albensis* M384^{T}. *Microbiology* **149**, 2227–2234 (2003).
24. K. Makrilakis, The role of DPP-4 inhibitors in the treatment algorithm of type 2 diabetes mellitus: When to select, what to expect. *Int. J. Environ. Res. Public Health* **16**, 2720 (2019).
25. Q. Cheng, J. Cheng, D. Cordato, J. Gao, Can dipeptidyl peptidase-4 inhibitors treat cognitive disorders? *Pharmacol. Ther.* **212**, 107559 (2020).
26. Y. Dumont, A. Fournier, R. Quirion, Expression and characterization of the neuropeptide Y Y_5 receptor subtype in the rat brain. *J. Neurosci.* **18**, 5565–5574 (1998).
27. J. C. Morales-Medina, Y. Dumont, R. Quirion, A possible role of neuropeptide Y in depression and stress. *Brain Res.* **1314**, 194–205 (2010).
28. P. Holzer, F. Reichmann, A. Farzi, Neuropeptide Y, peptide YY and pancreatic polypeptide in the gut–brain axis. *Neuropeptides* **46**, 261–274 (2012).
29. P. Datar, S. Srivastava, E. Coutinho, G. Govil, Substance P: Structure, function, and therapeutics. *Curr. Top. Med. Chem.* **4**, 75–103 (2004).
30. C. Severini, C. Petrella, P. Calissano, Substance P and Alzheimer's disease: Emerging novel roles. *Curr. Alzheimer Res.* **13**, 964–972 (2016).
31. S. Ahmad, L. Wang, P. E. Ward, Dipeptidyl (amino) peptidase IV and aminopeptidase M metabolize circulating substance P in vivo. *J. Pharmacol. Exp. Ther.* **260**, 1257–1261 (1992).
32. T. M. O'Connor, J. O'Connell, D. I. O'Brien, T. Goode, C. P. Bredin, F. Shanahan, The role of substance P in inflammatory disease. *J. Cell. Physiol.* **201**, 167–180 (2004).
33. P. Rigolet, X. G. Xi, S. Rety, J. F. Chich, The structural comparison of the bacterial PepX and human DPP-IV reveals sites for the design of inhibitors of PepX activity. *FEBS J.* **272**, 2050–2059 (2005).
34. M. Olivares, P. Hernández-Calderón, S. Cárdenas-Brito, R. Liébana-García, Y. Sanz, A. Benítez-Páez, Gut microbiota DPP4-like enzymes are increased in type-2 diabetes and contribute to incretin inactivation. *Genome Biol.* **25**, 174 (2024).
35. R. J. Wallace, N. McKain, G. A. Broderick, L. M. Rode, N. D. Walker, C. J. Newbold, J. Kopecny, Peptidases of the rumen bacterium, *Prevotella ruminicola*. *Anaerobe* **3**, 35–42 (1997).
36. D. Fteita, A. A. Musrati, E. Könönen, X. Ma, M. Gürsoy, M. Peurla, E. Söderling, H. O. Sintim, U. K. Gürsoy, Dipeptidyl peptidase IV and quorum sensing signaling in biofilm-related virulence of *Prevotella aurantiaca*. *Anaerobe* **48**, 152–159 (2017).
37. T.-K. Dao, T.-H. Do, N.-G. Le, H.-D. Nguyen, T.-Q. Nguyen, T.-T.-H. Le, N.-H. Truong, Understanding the Role of *Prevotella* genus in the digestion of Lignocellulose and other substrates in vietnamese native Goats' Rumen by metagenomic deep sequencing. *Animals* **11**, 3257 (2021).
38. N. Larsen, F. K. Vogensen, F. W. Van Den Berg, D. S. Nielsen, A. S. Andreasen, B. K. Pedersen, W. A. Al-Soud, S. J. Sørensen, L. H. Hansen, M. Jakobsen, Gut microbiota in human adults with type 2 diabetes differs from non-diabetic adults. *PLOS ONE* **5**, e9085 (2010).
39. J.-P. Furet, L.-C. Kong, J. Tap, C. Poitou, A. Basdevant, J.-L. Bouillot, D. Mariat, G. Corthier, J. Doré, C. Henegar, Differential adaptation of human gut microbiota to bariatric surgery–induced weight loss: Links with metabolic and low-grade inflammation markers. *Diabetes* **59**, 3049–3057 (2010).
40. M. Guo, J. Peng, X. Huang, L. Xiao, F. Huang, Z. Zuo, Gut microbiome features of Chinese patients newly diagnosed with Alzheimer's disease or mild cognitive impairment. *J. Alzheimers Dis.* **80**, 299–310 (2021).
41. M. R. Aljumaah, U. Bhatia, J. Roach, J. Gunstad, M. A. A. Peril, The gut microbiome, mild cognitive impairment, and probiotics: A randomized clinical trial in middle-aged and older adults. *Clin. Nutr.* **41**, 2565–2576 (2022).
42. H. Rong, X.-H. Xie, J. Zhao, W.-T. Lai, M.-B. Wang, D. Xu, Y.-H. Liu, Y.-Y. Guo, S.-X. Xu, W.-F. Deng, Similarly in depression, nuances of gut microbiota: Evidences from a shotgun metagenomics sequencing study on major depressive disorder versus bipolar disorder with current major depressive episode patients. *J. Psychiatr. Res.* **113**, 90–99 (2019).
43. J. S. Bajaj, A. Shamsaddini, A. Fagan, S. McGeorge, E. Gavis, M. Sikaroodi, L. A. Brenner, J. B. Wade, P. M. Gillevet, Distinct gut microbial compositional and functional changes associated with impaired inhibitory control in patients with cirrhosis. *Gut Microbes* **13**, 1953247 (2021).
44. D.-W. Kang, J. G. Park, Z. E. Ilhan, G. Wallstrom, J. LaBaer, J. B. Adams, R. Krajmalnik-Brown, Reduced incidence of *Prevotella* and other fermenters in intestinal microflora of autistic children. *PLOS ONE* **8**, e68322 (2013).
45. T. Kato, T. Nagatsu, K. Fukasawa, M. Harada, I. Nagatsu, S. Sakakibara, Successive cleavage of N-terminal Arg1-Pro2 and Lys3-Pro4 from substance P but no release of Arg1-Pro2 from bradykinin, by X-Pro dipeptidyl-aminopeptidase. *Biochim. Biophys. Acta* **525**, 417–422 (1978).
46. C. F. Deacon, A. H. Johnsen, J. J. Holst, Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. *J. Clin. Endocrinol. Metab.* **80**, 952–957 (1995).
47. C. Sun, Y. Xiao, J. Li, B. Ge, X. Chen, H. Liu, T. Zheng, Nonenzymatic function of DPP4 in diabetes-associated mitochondrial dysfunction and cognitive impairment. *Alzheimers Dement.* **18**, 966–987 (2022).
48. C. Klemann, L. Wagner, M. Stephan, S. von Hörsten, Cut to the chase: A review of CD26/dipeptidyl peptidase-4's (DPP4) entanglement in the immune system. *Clin. Exp. Immunol.* **185**, 1–21 (2016).
49. W. A. Weihofen, J. Liu, W. Reutter, W. Saenger, H. Fan, Crystal structure of CD26/dipeptidyl-peptidase IV in complex with adenosine deaminase reveals a highly amphiphilic interface. *J. Biol. Chem.* **279**, 43330–43335 (2004).
50. H. Fan, F. L. Tansi, W. A. Weihofen, C. Böttcher, J. Hu, J. Martinez, W. Saenger, W. Reutter, Molecular mechanism and structural basis of interactions of dipeptidyl peptidase IV with adenosine deaminase and human immunodeficiency virus type-1 transcription transactivator. *Eur. J. Cell Biol.* **91**, 265–273 (2012).
51. K. Ohnuma, M. Uchiyama, T. Yamochi, K. Nishibashi, O. Hosono, N. Takahashi, S. Kina, H. Tanaka, X. Lin, N. H. Dang, Caveolin-1 triggers T-cell activation via CD26 in association with CARMA1. *J. Biol. Chem.* **282**, 10117–10131 (2007).

52. K. Ohnuma, M. Uchiyama, R. Hatano, W. Takasawa, Y. Endo, N. H. Dang, C. Morimoto, Blockade of CD26-mediated T cell costimulation with soluble caveolin-1-Ig fusion protein induces anergy in CD4+ T cells. *Biochem. Biophys. Res. Commun.* **386**, 327–332 (2009).
53. J. Zhong, X. Rao, S. Rajagopalan, An emerging role of dipeptidyl peptidase 4 (DPP4) beyond glucose control: Potential implications in cardiovascular disease. *Atherosclerosis* **226**, 305–314 (2013).
54. K. Loster, K. Zeilinger, D. Schuppan, W. Reutter, The cysteine-rich region of dipeptidyl peptidase IV (CD 26) is the collagen binding site. *Biochem. Biophys. Res. Commun.* **217**, 341–348 (1995).
55. J. Zhong, Q. Gong, A. Goud, S. Srinivasamaharaj, S. Rajagopalan, Recent advances in dipeptidyl-peptidase-4 inhibition therapy: Lessons from the bench and clinical trials. *J. Diabetes Res.* **2015**, 606031 (2015).
56. D. Röhrborn, N. Wronkowitz, J. Eckel, DPP4 in diabetes. *Front. Immunol.* **6**, 386 (2015).
57. K. M. Chung, J. H. Cheng, C. S. Suen, C. H. Huang, C. H. Tsai, L. H. Huang, Y. R. Chen, A. H. J. Wang, W. T. Jiaang, M. J. Hwang, The dimeric transmembrane domain of prolyl dipeptidase DPP-IV contributes to its quaternary structure and enzymatic activities. *Protein Sci.* **19**, 1627–1638 (2010).
58. A.-M. Lambeir, C. Durinx, S. Scharpé, I. De Meester, Dipeptidyl-peptidase IV from bench to bedside: An update on structural properties, functions, and clinical aspects of the enzyme DPP IV. *Crit. Rev. Clin. Lab. Sci.* **40**, 209–294 (2003).
59. K. Aertgeerts, S. Ye, M. G. Tennant, M. L. Kraus, J. Rogers, B. C. Sang, R. J. Skene, D. R. Webb, G. S. Prasad, Crystal structure of human dipeptidyl peptidase IV in complex with a decapeptide reveals details on substrate specificity and tetrahedral intermediate formation. *Protein Sci.* **13**, 412–421 (2004).
60. C. F. Deacon, Dipeptidyl peptidase-4 inhibitors in the treatment of type 2 diabetes: A comparative review. *Diabetes Obes. Metab.* **13**, 7–18 (2011).
61. D. Marguet, L. Baggio, T. Kobayashi, A.-M. Bernard, M. Pierres, P. F. Nielsen, U. Ribel, T. Watanabe, D. J. Drucker, N. Wagtmann, Enhanced insulin secretion and improved glucose tolerance in mice lacking CD26. *Proc. Natl. Acad. Sci. U.S.A.* **97**, 6874–6879 (2000).
62. Y. Ohara-Nemoto, M. Nakasato, Y. Shimoyama, T. T. Baba, T. Kobayakawa, T. Ono, T. Yaegashi, S. Kimura, T. K. Nemoto, Degradation of incretins and modulation of blood glucose levels by periodontopathic bacterial dipeptidyl peptidase 4. *Infect. Immun.* **85**, e00277–e00217 (2017).
63. A. K. Sterkel, J. L. Lorenzini, J. S. Fites, K. S. Vignesh, T. D. Sullivan, M. Wuthrich, T. Brandhorst, N. Hernandez-Santos, G. S. Deepe, B. S. Klein, Fungal mimicry of a mammalian aminopeptidase disables innate immunity and promotes pathogenicity. *Cell Host Microbe* **19**, 361–374 (2016).
64. S. Vermout, A. Baldo, J. Tabart, B. Losson, B. Mignon, Secreted dipeptidyl peptidases as potential virulence factors for *Microsporum canis*. *FEMS Immunol. Med. Microbiol.* **54**, 299–308 (2008).
65. A. Mathy, A. Baldo, L. Schoofs, L. Cambier, V. Defaweux, J. Tabart, F. Maréchal, F. Symoens, B. Mignon, Fungalysin and dipeptidyl-peptidase gene transcription in *Microsporum canis* strains isolated from symptomatic and asymptomatic cats. *Vet. Microbiol.* **146**, 179–182 (2010).
66. M. Monod, B. Lechenne, O. Jousson, D. Grand, C. Zaugg, R. Stocklin, E. Grouzmann, Aminopeptidases and dipeptidyl-peptidases secreted by the dermatophyte *Trichophyton rubrum*. *Microbiology* **151**, 145–155 (2005).
67. R. Stentz, E. Jones, R. Juodeikis, U. Wegmann, M. Guirro, A. J. Goldson, A. Brion, C. Booth, P. Sudhakar, I. R. Brown, T. Korcsmáros, S. R. Carding, The proteome of extracellular vesicles produced by the human gut bacteria *Bacteroides thetaiotaomicron* in vivo is influenced by environmental and host-derived factors. *Appl. Environ. Microbiol.* **88**, e00533–e00522 (2022).
68. D. Rea, R. Van Elzen, H. De Winter, S. Van Goethem, B. Landuyt, W. Luyten, L. Schoofs, P. Van Der Veken, K. Augustyns, I. De Meester, V. Fülöp, A.-M. Lambeir, Crystal structure of *Porphyromonas gingivalis* dipeptidyl peptidase 4 and structure-activity relationships based on inhibitor profiling. *Eur. J. Med. Chem.* **139**, 482–491 (2017).
69. Y. Nakajima, K. Ito, T. Toshima, T. Egawa, H. Zheng, H. Oyama, Y.-F. Wu, E. Takahashi, K. Kyono, T. Yoshimoto, Dipeptidyl aminopeptidase IV from *Stenotrophomonas maltophilia* exhibits activity against a substrate containing a 4-hydroxyproline residue. *J. Bacteriol.* **190**, 7819–7829 (2008).
70. S. Roppongi, Y. Suzuki, C. Tateoka, M. Fujimoto, S. Morisawa, I. Iizuka, A. Nakamura, N. Honma, Y. Shida, W. Ogasawara, N. Tanaka, Y. Sakamoto, T. Nonaka, Crystal structures of a bacterial dipeptidyl peptidase IV reveal a novel substrate recognition mechanism distinct from that of mammalian orthologues. *Sci. Rep.* **8**, 2714 (2018).
71. Y. Koreeda, M. Hayakawa, T. Ikemi, Y. Abiko, Isolation and characterisation of dipeptidyl peptidase IV from *Prevotella loescheii* ATCC 15930. *Arch. Oral Biol.* **46**, 759–766 (2001).
72. D. Grenier, P. Gauthier, P. Plamondon, K. Nakayama, D. Mayrand, Studies on the aminopeptidase activities of *Porphyromonas gingivalis*. *Oral Microbiol. Immunol.* **16**, 212–217 (2001).
73. Y. Ohara-Nemoto, Y. Shimoyama, T. Ono, M. T. Sarwar, M. Nakasato, M. Sasaki, T. K. Nemoto, Expanded substrate specificity supported by P1′ and P2′ residues enables bacterial dipeptidyl-peptidase 7 to degrade bioactive peptides. *J. Biol. Chem.* **298**, 101585 (2022).
74. L. J. Keller, T. H. Nguyen, L. J. Liu, B. M. Hurysz, M. Lakemeyer, M. Guerra, D. J. Gelsinger, R. Chanin, N. Ngo, K. M. Lum, Chemoproteomic identification of a DPP4 homolog in *Bacteroides thetaiotaomicron*. *Nat. Chem. Biol.* **19**, 1469–1479 (2023).
75. F. B. Teixeira, M. T. Saito, F. C. Matheus, R. D. Prediger, E. S. Yamada, C. S. Maia, R. R. Lima, Periodontitis and Alzheimer's disease: A possible comorbidity between oral chronic inflammatory condition and neuroinflammation. *Front. Aging Neurosci.* **9**, 327 (2017).
76. B. von Troil-Lindén, H. Torkko, S. Alaluusua, H. Jousimies-Somer, S. Asikainen, Salivary levels of suspected periodontal pathogens in relation to periodontal status and treatment. *J. Dent. Res.* **74**, 1789–1795 (1995).
77. I. Saygun, N. Nizam, I. Keskiner, V. Bal, A. Kubar, C. Açıkel, M. Serdar, J. Slots, Salivary infectious agents and periodontal disease status. *J. Periodontal Res.* **46**, 235–239 (2011).
78. M. Y. Walker, S. Pratap, J. H. Southerland, C. M. Farmer-Dixon, K. Lakshmyya, P. R. Gangula, Role of oral and gut microbiome in nitric oxide-mediated colon motility. *Nitric Oxide* **73**, 81–88 (2018).
79. I. Olsen, K. Yamazaki, Can oral bacteria affect the microbiome of the gut? *J. Oral Microbiol.* **11**, 1586422 (2019).
80. K. Sato, N. Takahashi, T. Kato, Y. Matsuda, M. Yokoji, M. Yamada, T. Nakajima, N. Kondo, N. Endo, R. Yamamoto, Aggravation of collagen-induced arthritis by orally administered *Porphyromonas gingivalis* through modulation of the gut microbiota and gut immune system. *Sci. Rep.* **7**, 6955 (2017).
81. S. Ahmadi, A. Razazan, R. Nagpal, S. Jain, B. Wang, S. P. Mishra, S. Wang, J. Justice, J. Ding, D. A. McClain, Metformin reduces aging-related leaky gut and improves cognitive function by beneficially modulating gut microbiome/goblet cell/mucin axis. *J. Gerontol. A Biol. Sci. Med. Sci.* **75**, e9–e21 (2020).
82. A. Peters, H. Wekerle, Autoimmune diabetes mellitus and the leaky gut. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 14788–14790 (2019).
83. R. H. Ahmed, H. Z. Huri, S. Muniandy, Z. Al-Hamodi, B. Al-Absi, A. Alsalahi, M. F. Razif, Altered circulating concentrations of active glucagon-like peptide (GLP-1) and dipeptidyl peptidase 4 (DPP4) in obese subjects and their association with insulin resistance. *Clin. Biochem.* **50**, 746–749 (2017).
84. T. F. Teixeira, M. C. Collado, C. L. Ferreira, J. Bressan, M. do Carmo G Peluzio, Potential mechanisms for the emerging link between obesity and increased intestinal permeability. *Nutr. Res.* **32**, 637–647 (2012).
85. H. K. Pedersen, V. Gudmundsdottir, H. B. Nielsen, T. Hyotylainen, T. Nielsen, B. A. H. Jensen, K. Forslund, F. Hildebrand, E. Prifti, G. Falony, E. L. Chatelier, F. Levenez, J. Doré, I. Mattila, D. R. Plichta, P. Pöhö, L. I. Hellgren, M. Arumugam, S. Sunagawa, S. Vieira-Silva, T. Jørgensen, J. B. Holm, K. Trošt, Meta HIT Consortium, K. Kristiansen, S. Brix, J. Raes, J. Wang, T. Hansen, P. Bork, S. Brunak, M. Oresic, S. D. Ehrlich, O. Pedersen, Human gut microbes impact host serum metabolome and insulin sensitivity. *Nature* **535**, 376–381 (2016).
86. P. Kovatcheva-Datchary, A. Nilsson, R. Akrami, Y. S. Lee, F. De Vadder, T. Arora, A. Hallen, E. Martens, I. Björck, F. Bäckhed, Dietary fiber-induced improvement in glucose metabolism is associated with increased abundance of *Prevotella*. *Cell Metab.* **22**, 971–982 (2015).
87. V. E. Sanborn, M. A. Azcarate-Peril, J. Gunstad, *Lactobacillus rhamnosus* GG and HbA1c in middle age and older adults without type 2 diabetes mellitus: A preliminary randomized study. *Diabetes Metab. Syndr. Clin. Res. Rev.* **14**, 907–909 (2020).
88. V. Sanborn, M. A. Azcarate-Peril, J. Updegraff, L. M. Manderino, J. Gunstad, A randomized clinical trial examining the impact of LGG probiotic supplementation on psychological status in middle-aged and older adults. *Contemp. Clin. Trials Commun.* **12**, 192–197 (2018).
89. W. Creutzfeldt, The incretin concept today. *Diabetologia* **16**, 75–85 (1979).
90. D. J. Drucker, M. A. Nauck, The incretin system: Glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* **368**, 1696–1705 (2006).
91. J. Doupis, A. Veves, DPP4 inhibitors: A new approach in diabetes treatment. *Adv. Ther.* **25**, 627–643 (2008).
92. G. Herman, P. Stein, N. Thornberry, J. Wagner, Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: Focus on sitagliptin. *Clin. Pharmacol. Ther.* **81**, 761–767 (2007).
93. A. Scheen, DPP-4 inhibitors in the management of type 2 diabetes: A critical review of head-to-head trials. *Diabetes Metab.* **38**, 89–101 (2012).
94. P. Balakumar, S. A. Dhanaraj, Cardiovascular pleiotropic actions of DPP-4 inhibitors: A step at the cutting edge in understanding their additional therapeutic potentials. *Cell. Signal.* **25**, 1799–1803 (2013).
95. G. Coppolino, C. Leporini, L. Rivoli, F. Ursini, E. D. di Paola, V. Cernaro, F. Arturi, D. Bolignano, E. Russo, G. De Sarro, M. Andreucci, Exploring the effects of DPP-4 inhibitors on the kidney from the bench to clinical trials. *Pharmacol. Res.* **129**, 274–294 (2018).
96. S. Huang, Y. Huang, W. Lin, L. Wang, Y. Yang, P. Li, L. Xiao, Y. Chen, Q. Chu, X. Yuan, Sitagliptin alleviates radiation-induced intestinal injury by activating NRF2-antioxidant axis, mitigating NLRP3 Inf--lammasome activation, and reversing gut microbiota disorder. *Oxid. Med. Cell. Longev.* **2022**, 2586305 (2022).

97. B. Li, Y. R. Luo, Q. Zhang, S. H. Fu, Y. Dai Chen, J. W. Tian, Y. Guo, Sitagliptin, a dipeptidyl peptidase-4 inhibitor, attenuates apoptosis of vascular smooth muscle cells and reduces atherosclerosis in diabetic apolipoprotein E–deficient mice. *Vascul. Pharmacol.* **140**, 106854 (2021).
98. M. Trocha, M. G. Fleszar, P. Fortuna, Ł. Lewandowski, K. Gostomska-Pampuch, T. Sozański, A. Merwid-Ląd, M. Krzystek-Korpacka, Sitagliptin modulates oxidative, nitrative and halogenative stress and inflammatory response in rat model of hepatic ischemia-reperfusion. *Antioxidants* **10**, 1168 (2021).
99. S. Chen, M. Zhou, J. Sun, A. Guo, R. L. Fernando, Y. Chen, P. Peng, G. Zhao, Y. Deng, DPP-4 inhibitor improves learning and memory deficits and AD-like neurodegeneration by modulating the GLP-1 signaling. *Neuropharmacology* **157**, 107668 (2019).
100. H. H. Arab, A. H. Eid, A. M. Mahmoud, M. A. Senousy, Linagliptin mitigates experimental inflammatory bowel disease in rats by targeting inflammatory and redox signaling. *Life Sci.* **273**, 119295 (2021).
101. X.-Y. Yuan, X.-G. Wang, Mild cognitive impairment in type 2 diabetes mellitus and related risk factors: A review. *Rev. Neurosci.* **28**, 715–723 (2017).
102. J. Janson, T. Laedtke, J. E. Parisi, P. O'Brien, R. C. Petersen, P. C. Butler, Increased risk of type 2 diabetes in Alzheimer disease. *Diabetes* **53**, 474–481 (2004).
103. G. Hu, P. Jousilahti, S. Bidel, R. Antikainen, J. Tuomilehto, Type 2 diabetes and the risk of Parkinson's disease. *Diabetes Care* **30**, 842–847 (2007).
104. L. A. Farrer, Diabetes mellitus in Huntington disease. *Clin. Genet.* **27**, 62–67 (1985).
105. S. Ahtiluoto, T. Polvikoski, M. Peltonen, A. Solomon, J. Tuomilehto, B. Winblad, R. Sulkava, M. Kivipelto, Diabetes, Alzheimer disease, and vascular dementia: A population-based neuropathologic study. *Neurology* **75**, 1195–1202 (2010).
106. S. L. Conarello, Z. Li, J. Ronan, R. S. Roy, L. Zhu, G. Jiang, F. Liu, J. Woods, E. Zycband, D. E. Moller, Mice lacking dipeptidyl peptidase IV are protected against obesity and insulin resistance. *Proc. Natl. Acad. Sci. U.S.A.* **100**, 6825–6830 (2003).
107. J. Kosaraju, R. Holsinger, L. Guo, K. Y. Tam, Linagliptin, a dipeptidyl peptidase-4 inhibitor, mitigates cognitive deficits and pathology in the 3xTg-AD mouse model of Alzheimer's disease. *Mol. Neurobiol.* **54**, 6074–6084 (2017).
108. A. Kask, J. Harro, S. von Hörsten, J. P. Redrobe, Y. Dumont, R. Quirion, The neurocircuitry and receptor subtypes mediating anxiolytic-like effects of neuropeptide Y. *Neurosci. Biobehav. Rev.* **26**, 259–283 (2002).
109. M. Heilig, The NPY system in stress, anxiety and depression. *Neuropeptides* **38**, 213–224 (2004).
110. A. M. Borzì, G. Condorelli, A. Biondi, F. Basile, E. S. D. Vicari, C. Buscemi, S. Luca, M. Vacante, Effects of vildagliptin, a DPP-4 inhibitor, in elderly diabetic patients with mild cognitive impairment. *Arch. Gerontol. Geriatr.* **84**, 103896 (2019).
111. Y. Hu, J. Wang, J. Wang, W. Chen, Q. Zhang, DPP-4 inhibitor improved the cognitive function in diabetic rats. *J. Healthc. Eng.* **2022**, 8280389 (2022).
112. Z. Li, Y. Zhang, X. Meng, M. Li, W. Cao, J. Yang, X. Xu, W. Liu, W. Li, Q. Cai, A novel DPP-4 inhibitor Gramcyclin A attenuates cognitive deficits in APP/PS1/tau triple transgenic mice via enhancing brain GLP-1-dependent glucose uptake. *Phytother. Res.* **36**, 1297–1309 (2022).
113. S. Lim, M. Sohn, J. C. Florez, M. A. Nauck, J. Ahn, Effects of initial combinations of gemigliptin plus metformin compared with glimepiride plus metformin on gut microbiota and glucose regulation in obese patients with type 2 diabetes: The intestine study. *Nutrients* **15**, 248 (2023).
114. X. Liao, L. Song, B. Zeng, B. Liu, Y. Qiu, H. Qu, Y. Zheng, M. Long, H. Zhou, Y. Wang, Alteration of gut microbiota induced by DPP-4i treatment improves glucose homeostasis. *EBioMedicine* **44**, 665–674 (2019).
115. M. M. Smits, K. S. Fluitman, H. Herrema, M. Davids, M. H. Kramer, A. K. Groen, C. Belzer, W. M. de Vos, D. L. Cahen, M. Nieuwdorp, Liraglutide and sitagliptin have no effect on intestinal microbiota composition: A 12-week randomized placebo-controlled trial in adults with type 2 diabetes. *Diabetes Metab.* **47**, 101223 (2021).
116. S. Hiel, M. A. Gianfrancesco, J. Rodriguez, D. Portheault, Q. Leyrolle, L. B. Bindels, C. G. da Silveira Cauduro, M. D. Mulders, G. Zamariola, A.-S. Azzi, Link between gut microbiota and health outcomes in inulin-treated obese patients: Lessons from the Food4Gut multicenter randomized placebo-controlled trial. *Clin. Nutr.* **39**, 3618–3628 (2020).
117. K. Forslund, F. Hildebrand, T. Nielsen, G. Falony, E. L. Chatelier, S. Sunagawa, E. Prifti, S. Vieira-Silva, V. Gudmundsdottir, H. K. Pedersen, M. Arumugam, K. Kristiansen, A. Y. Voigt, H. Vestergaard, R. Hercog, P. I. Costea, J. R. Kultima, J. Li, T. Jørgensen, F. Levenez, J. Dore, Meta HIT consortium, H. B. Nielsen, S. Brunak, J. Raes, T. Hansen, J. Wang, S. D. Ehrlich, P. Bork, O. Pedersen, Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. *Nature* **528**, 262–266 (2015).
118. H. Wu, E. Esteve, V. Tremaroli, M. T. Khan, R. Caesar, L. Mannerås-Holm, M. Ståhlman, L. M. Olsson, M. Serino, M. Planas-Fèlix, G. Xifra, J. M. Mercader, D. Torrents, R. Burcelin, W. Ricart, R. Perkins, J. M. Fernàndez-Real, F. Bäckhed, Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. *Nat. Med.* **23**, 850–858 (2017).
119. J. De La Cuesta-Zuluaga, N. T. Mueller, V. Corrales-Agudelo, E. P. Velásquez-Mejía, J. A. Carmona, J. M. Abad, J. S. Escobar, Metformin is associated with higher relative abundance of mucin-degrading *Akkermansia muciniphila* and several short-chain fatty acid–producing microbiota in the gut. *Diabetes Care* **40**, 54–62 (2017).
120. N.-R. Shin, J.-C. Lee, H.-Y. Lee, M.-S. Kim, T. W. Whon, M.-S. Lee, J.-W. Bae, An increase in the *Akkermansia* spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. *Gut* **63**, 727–735 (2014).
121. L.-C. Chen, Z.-Y. Fan, H.-Y. Wang, D.-C. Wen, S.-Y. Zhang, Effect of polysaccharides from adlay seed on anti-diabetic and gut microbiota. *Food Funct.* **10**, 4372–4380 (2019).
122. X. Zhang, Y. Zhao, J. Xu, Z. Xue, M. Zhang, X. Pang, X. Zhang, L. Zhao, Modulation of gut microbiota by berberine and metformin during the treatment of high-fat diet-induced obesity in rats. *Sci. Rep.* **5**, 14405 (2015).
123. H.-X. Cui, L.-S. Zhang, Y. Luo, K. Yuan, Z.-Y. Huang, Y. Guo, A purified anthraquinone-glycoside preparation from rhubarb ameliorates type 2 diabetes mellitus by modulating the gut microbiota and reducing inflammation. *Front. Microbiol.* **10**, 1423 (2019).
124. Q. Zhang, N. Hu, Effects of metformin on the gut microbiota in obesity and type 2 diabetes mellitus. *Diabetes Metab. Syndr. Obes.* **13**, 5003–5014 (2020).
125. J. Mo, J. Ni, M. Zhang, Y. Xu, Y. Li, N. Karim, W. Chen, Mulberry anthocyanins ameliorate DSS-induced ulcerative colitis by improving intestinal barrier function and modulating gut microbiota. *Antioxidants* **11**, 1674 (2022).
126. J. R. Turner, Intestinal mucosal barrier function in health and disease. *Nat. Rev. Immunol.* **9**, 799–809 (2009).
127. J. Gao, Y. Li, Y. Wan, T. Hu, L. Liu, S. Yang, Z. Gong, Q. Zeng, Y. Wei, W. Yang, Z. Zeng, X. He, S.-H. Huang, H. Cao, A novel postbiotic from *Lactobacillus rhamnosus* GG with a beneficial effect on intestinal barrier function. *Front. Microbiol.* **10**, 477 (2019).
128. C. S. Eun, Y. S. Kim, D. S. Han, J. H. Choi, A. R. Lee, Y. K. Park, *Lactobacillus casei* prevents impaired barrier function in intestinal epithelial cells. *APMIS* **119**, 49–56 (2011).
129. X. Yan, B. Feng, P. Li, Z. Tang, L. Wang, Microflora disturbance during progression of glucose intolerance and effect of sitagliptin: An animal study. *J. Diabetes Res.* **2016**, 2093171 (2016).
130. Y. Chang, T. Xiang, H. Wang, F. Zhou, J. Liang, W. Deng, S. Li, W. Chen, K. Li, Y. Li, The DPP-4 inhibitor sitagliptin alters the gut microbiota and prevents neuronal damage of hippocampus in STZ induced type 2 diabetic rats. Research Sequare [Preprint] (2020). https://doi.org/10.21203/rs.2.21932/v1.
131. L. Wang, P. Li, Z. Tang, X. Yan, B. Feng, Structural modulation of the gut microbiota and the relationship with body weight: Compared evaluation of liraglutide and saxagliptin treatment. *Sci. Rep.* **6**, 33251 (2016).
132. M. A. Nauck, I. Vardarli, Genetic determinants predicting efficacy of glucose-lowering drugs? *Diabetes Care* **33**, 2123–2125 (2010).
133. I. Tkáč, I. Gotthardová, Pharmacogenetic aspects of the treatment of type 2 diabetes with the incretin effect enhancers. *Pharmacogenomics* **17**, 795–804 (2016).
134. M. Olivares, A. M. Neyrinck, S. A. Pötgens, M. Beaumont, N. Salazar, P. D. Cani, L. B. Bindels, N. M. Delzenne, The DPP-4 inhibitor vildagliptin impacts the gut microbiota and prevents disruption of intestinal homeostasis induced by a Western diet in mice. *Diabetologia* **61**, 1838–1848 (2018).
135. G. Tolhurst, H. Heffron, Y. S. Lam, H. E. Parker, A. M. Habib, E. Diakogiannaki, J. Cameron, J. Grosse, F. Reimann, F. M. Gribble, Short-chain fatty acids stimulate glucagon-like peptide-1 secretion via the G-protein–coupled receptor FFAR2. *Diabetes* **61**, 364–371 (2012).
136. L. E. Carpio, M. Olivares, A. Benítez-Paez, E. Serrano-Candelas, S. J. Barigye, Y. Sanz, R. Gozalbes, Comparative binding study of gliptins to bacterial DPP4-like enzymes for the treatment of type 2 diabetes mellitus (T2DM). *Int. J. Mol. Sci.* **25**, 5744 (2024).
137. H. Panwar, D. Calderwood, I. R. Grant, S. Grover, B. D. Green, Lactobacilli possess inhibitory activity against dipeptidyl peptidase-4 (DPP-4). *Ann. Microbiol.* **66**, 505–509 (2016).
138. T. D. Filippatos, V. G. Athyros, M. S. Elisaf, The pharmacokinetic considerations and adverse effects of DDP-4 inhibitors. *Expert Opin. Drug Metab. Toxicol.* **10**, 787–812 (2014).
139. H. Umezawa, T. Aoyagi, K. Ogawa, H. Naganawa, M. Hamada, T. Takeuchi, Diprotins A and B, inhibitors of dipeptidyl aminopeptidase IV, produced by bacteria. *J. Antibiot.* **37**, 422–425 (1984).
140. H. Umezawa, Studies on low-molecular-weight immunomodifiers produced by microorganisms: Results of ten years' effort. *Rev. Infect. Dis.* **6**, 412–420 (1984).
141. M. Abe, T. Akiyama, Y. Umezawa, K. Yamamoto, M. Nagai, H. Yamazaki, Y.-I. Ichikawa, Y. Muraoka, Synthesis and biological activity of sulphostin analogues, novel dipeptidyl peptidase IV inhibitors. *Bioorg. Med. Chem.* **13**, 785–797 (2005).
142. Z. Zeng, J. Luo, F. Zuo, Y. Zhang, H. Ma, S. Chen, Screening for potential novel probiotic *Lactobacillus* strains based on high dipeptidyl peptidase IV and α-glucosidase inhibitory activity. *J. Funct. Foods* **20**, 486–495 (2016).
143. F. Yan, N. Li, Y. Yue, C. Wang, L. Zhao, S. E. Evivie, B. Li, G. Huo, Screening for potential novel probiotics with dipeptidyl peptidase IV-inhibiting activity for type 2 diabetes attenuation in vitro and in vivo. *Front. Microbiol.* **10**, 2855 (2020).
144. M. Liu, J. R. Bayjanov, B. Renckens, A. Nauta, R. J. Siezen, The proteolytic system of lactic acid bacteria revisited: A genomic comparison. *BMC Genomics* **11**, 36 (2010).

145. J. Sha, J. Song, Y. Huang, Y. Zhang, H. Wang, Y. Zhang, H. Suo, Inhibitory effect and potential mechanism of *Lactobacillus plantarum* YE4 against dipeptidyl peptidase-4. *Foods* **11**, 80 (2021).
146. R. Xiao, R. Wang, S. Li, X. Kang, Y. Ren, E. Sun, C. Wang, J. He, J. Zhan, Preliminary evaluation of potential properties of three probiotics and their combination with prebiotics on GLP-1 secretion and type 2 diabetes alleviation. *J. Food Qual.* **2022**, 8586843 (2022).
147. M. A. Nauck, J. J. Meier, Incretin hormones: Their role in health and disease. *Diabetes Obes. Metab.* **20**, 5–21 (2018).
148. J. J. Meier, A. Gethmann, M. A. Nauck, O. Götze, F. Schmitz, C. F. Deacon, B. Gallwitz, W. E. Schmidt, J. J. Holst, The glucagon-like peptide-1 metabolite GLP-1-(9–36) amide reduces postprandial glycemia independently of gastric emptying and insulin secretion in humans. *Am. J. Physiol. Endocrinol. Metab.* **290**, E1118–E1123 (2006).
149. S. Paternoster, M. Falasca, Dissecting the physiology and pathophysiology of glucagon-like peptide-1. *Front. Endocrinol.* **9**, 584 (2018).
150. R. Mentlein, Mechanisms underlying the rapid degradation and elimination of the incretin hormones GLP-1 and GIP. *Best Pract. Res. Clin. Endocrinol. Metab.* **23**, 443–452 (2009).
151. E. Grouzmann, M. Monod, B. Landis, S. Wilk, N. Brakch, K. Nicoucar, R. Giger, D. Malis, I. Szalay-Quinodoz, C. Cavadas, D. R. Morel, J.-S. Lacroix, Loss of dipeptidylpeptidase IV activity in chronic rhinosinusitis contributes to the neurogenic inflammation induced by substance P in the nasal mucosa. *FASEB J.* **16**, 1132–1134 (2002).
152. C. Deacon, Circulation and degradation of GIP and GLP-1. *Horm. Metab. Res.* **36**, 761–765 (2004).
153. E. J. Jones, C. Booth, S. Fonseca, A. Parker, K. Cross, A. Miquel-Clopés, I. Hautefort, U. Mayer, T. Wileman, R. Stentz, S. R. Carding, The uptake, trafficking, and biodistribution of *Bacteroides thetaiotaomicron* generated outer membrane vesicles. *Front. Microbiol.* **11**, 57 (2020).
154. E. Jones, R. Stentz, A. Telatin, G. M. Savva, C. Booth, D. Baker, S. Rudder, S. C. Knight, A. Noble, S. R. Carding, The origin of plasma-derived bacterial extracellular vesicles in healthy individuals and patients with inflammatory bowel disease: A pilot study. *Genes* **12**, 1636 (2021).
155. C. Grau-Bové, C. González-Quilen, G. Cantini, P. Nardini, B. Espina, D. Bani, X. Terra, M. Blay, E. Rodríguez-Gallego, M. Luconi, GLP1 exerts paracrine activity in the intestinal lumen of human colon. *Int. J. Mol. Sci.* **23**, 3523 (2022).
156. L. J. Stevens, M. M. van Lipzig, S. L. Erpelinck, A. Pronk, J. van Gorp, H. M. Wortelboer, E. van de Steeg, A higher throughput and physiologically relevant two-compartmental human ex vivo intestinal tissue system for studying gastrointestinal processes. *Eur. J. Pharm. Sci.* **137**, 104989 (2019).
157. D. J. Drucker, The biology of incretin hormones. *Cell Metab.* **3**, 153–165 (2006).
158. D. J. Drucker, J. Philippe, S. Mojsov, W. L. Chick, J. F. Habener, Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc. Natl. Acad. Sci. U.S.A.* **84**, 3434–3438 (1987).
159. H. J. Grill, A role for GLP-1 in treating hyperphagia and obesity. *Endocrinology* **161**, bqaa093 (2020).
160. J. J. Meier, GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat. Rev. Endocrinol.* **8**, 728–742 (2012).
161. L. L. Baggio, D. J. Drucker, Biology of incretins: GLP-1 and GIP. *Gastroenterology* **132**, 2131–2157 (2007).
162. K. M. Heppner, M. Kirigiti, A. Secher, S. J. Paulsen, R. Buckingham, C. Pyke, L. B. Knudsen, N. Vrang, K. L. Grove, Expression and distribution of glucagon-like peptide-1 receptor mRNA, protein and binding in the male nonhuman primate (*Macaca mulatta*) brain. *Endocrinology* **156**, 255–267 (2015).
163. D. S. Kim, H.-I. Choi, Y. Wang, Y. Luo, B. J. Hoffer, N. H. Greig, A new treatment strategy for Parkinson's disease through the gut–brain axis: The glucagon-like peptide-1 receptor pathway. *Cell Transplant.* **26**, 1560–1571 (2017).
164. W.-H. Tsai, F.-C. Sung, L.-T. Chiu, Y.-H. Shih, M.-C. Tsai, S.-I. Wu, Decreased risk of anxiety in diabetic patients receiving glucagon-like peptide-1 receptor agonist: A nationwide, population-based cohort study. *Front. Pharmacol.* **13**, (2022).
165. N. García-Casares, G. González-González, C. de la Cruz-Cosme, F. J. Garzón-Maldonado, C. de Rojas-Leal, M. J. Ariza, M. Narváez, M. Á. Barbancho, J. A. García-Arnés, F. J. Tinahones, Effects of GLP-1 receptor agonists on neurological complications of diabetes. *Rev. Endocr. Metab. Disord.* **24**, 655–672 (2023).
166. C. Chimerel, E. Emery, D. K. Summers, U. Keyser, F. M. Gribble, F. Reimann, Bacterial metabolite indole modulates incretin secretion from intestinal enteroendocrine L cells. *Cell Rep.* **9**, 1202–1208 (2014).
167. M. Bogunovic, S. H. Davé, J. S. Tilstra, D. T. Chang, N. Harpaz, H. Xiong, L. F. Mayer, S. E. Plevy, Enteroendocrine cells express functional Toll-like receptors. *Am. J. Physiol. Gastrointest. Liver Physiol.* **292**, G1770–G1783 (2007).
168. L. Wang, X. Zhan, Z. Wang, J. Ma, X. Chang, X. Zhu, Toll-like receptor 4 is necessary for glucose-dependent glucagon-like peptide-1 secretion in male mice. *Biochem. Biophys. Res. Commun.* **510**, 104–109 (2019).
169. M. Ren, H. Zhang, J. Qi, A. Hu, Q. Jiang, Y. Hou, Q. Feng, O. Ojo, X. Wang, An almond-based low carbohydrate diet improves depression and glycometabolism in patients with type 2 diabetes through modulating gut microbiota and GLP-1: A randomized controlled trial. *Nutrients* **12**, 3036 (2020).
170. Y. Ma, E. Lee, H. Yoshikawa, T. Noda, J. Miyamoto, I. Kimura, R. Hatano, T. Miki, Phloretin suppresses carbohydrate-induced GLP-1 secretion via inhibiting short chain fatty acid release from gut microbiome. *Biochem. Biophys. Res. Commun.* **621**, 176–182 (2022).
171. J.-M. Zhang, Y.-S. Sun, L.-Q. Zhao, T.-T. Chen, M.-N. Fan, H.-C. Jiao, J.-P. Zhao, X.-J. Wang, F.-C. Li, H.-F. Li, H. Lin, SCFAs-induced GLP-1 secretion links the regulation of gut microbiome on hepatic lipogenesis in chickens. *Front. Microbiol.* **10**, 2176 (2019).
172. M. P. Yavropoulou, J. G. Yovos, Central regulation of glucose-dependent insulinotropic polypeptide secretion. *Vitam. Horm.* **84**, 185–201 (2010).
173. Z. Q. Zhang, C. Hölscher, GIP has neuroprotective effects in Alzheimer and Parkinson's disease models. *Peptides* **125**, 170184 (2020).
174. L. Zhang, C. Li, Z. Zhang, Z. Zhang, Q.-Q. Jin, L. Li, C. Hölscher, DA5-CH and semaglutide protect against neurodegeneration and reduce α-synuclein levels in the 6-OHDA Parkinson's Disease rat model. *Parkinsons Dis.* **2022**, 1428817 (2022).
175. T. S. Dong, W. Katzka, J. C. Yang, C. Chang, N. Arias-Jayo, V. Lagishetty, A. Balioukova, Y. Chen, E. Dutson, Z. Li, E. A. Mayer, J. R. Pisegna, C. Sanmiguel, J. P. Jacobs, Microbial changes from bariatric surgery alters glucose-dependent insulinotropic polypeptide and prevents fatty liver disease. *Gut Microbes* **15**, 2167170 (2023).
176. N. N. Kok, L. M. Morgan, C. M. Williams, M. B. Roberfroid, J.-P. Thissen, N. M. Delzenne, Insulin, glucagon-like peptide 1, glucose-dependent insulinotropic polypeptide and insulin-like growth factor I as putative mediators of the hypolipidemic effect of oligofructose in rats. *J. Nutr.* **128**, 1099–1103 (1998).
177. K. Tatemoto, Isolation and characterization of peptide YY (PYY), a candidate gut hormone that inhibits pancreatic exocrine secretion. *Proc. Natl. Acad. Sci. U.S.A.* **79**, 2514–2518 (1982).
178. R. Sandeva, S. Dimova, Z. Tsokeva, Neuropeptide Y family of peptides: Structure, anatomical expression, regulation, receptors and physiological functions. *Trakia J. Sci.* **5**, 8–15 (2007).
179. J. Redrobe, C. Carvajal, A. Kask, Y. Dumont, R. Quirion, "Neuropeptide Y and its receptor subtypes in the central nervous system: Emphasis on their role in animal models of psychiatric disorders" in *Neuropeptide Y and Related Peptides*, Handbook of Experimental Pharmacology, M. C. Michel, Ed. (Springer, 2004), vol. 162, pp. 101–136.
180. V. V. dos Santos, D. B. Santos, G. Lach, A. L. S. Rodrigues, M. Farina, T. C. De Lima, R. D. Prediger, Neuropeptide Y (NPY) prevents depressive-like behavior, spatial memory deficits and oxidative stress following amyloid-β (Aβ1–40) administration in mice. *Behav. Brain Res.* **244**, 107–115 (2013).
181. A. Thorsell, A. A. Mathé, Neuropeptide Y in alcohol addiction and affective disorders. *Front. Endocrinol.* **8**, 178 (2017).
182. J.-C. Martel, R. Alagar, Y. Robitaille, R. Quirion, Neuropeptide Y receptor binding sites in human brain. Possible alteration in Alzheimer's disease. *Brain Res.* **519**, 228–235 (1990).
183. S. Koide, H. Onishi, H. Hashimoto, T. Kai, S. Yamagami, Plasma neuropeptide Y is reduced in patients with Alzheimer's disease. *Neurosci. Lett.* **198**, 149–151 (1995).
184. J. Alom, R. Galard, R. Catalan, J. Castellanos, S. Schwartz, E. Tolosa, Cerebrospinal fluid neuropeptide Y in Alzheimer's disease. *Eur. Neurol.* **30**, 207–210 (1990).
185. R. Mentlein, P. Dahms, D. Grandt, R. Krüger, Proteolytic processing of neuropeptide Y and peptide YY by dipeptidyl peptidase IV. *Regul. Pept.* **49**, 133–144 (1993).
186. A. M. Hassan, G. Mancano, K. Kashofer, E. E. Frohlich, A. Matak, R. Mayerhofer, F. Reichmann, M. Olivares, A. M. Neyrinck, N. M. Delzenne, S. P. Claus, P. Holzer, High-fat diet induces depression-like behaviour in mice associated with changes in microbiome, neuropeptide Y, and brain metabolome. *Nutr. Neurosci.* **22**, 877–893 (2019).
187. Z. Wang, X. Zeng, Y. Mo, K. Smith, Y. Guo, J. Lin, Identification and characterization of a bile salt hydrolase from *Lactobacillus salivarius* for development of novel alternatives to antibiotic growth promoters. *Appl. Environ. Microbiol.* **78**, 8795–8802 (2012).
188. Z. Hafeez, C. Cakir-Kiefer, X. Lecomte, L. Miclo, A. Dary-Mourot, The X-prolyl dipeptidyl-peptidase PepX of *Streptococcus thermophilus* initially described as intracellular is also responsible for peptidase extracellular activity. *J. Dairy Sci.* **102**, 113–123 (2019).
189. Ö. Üstün-Aytekin, S. Arısoy, A. Ö. Aytekin, E. Yıldız, Statistical optimization of cell disruption techniques for releasing intracellular X-prolyl dipeptidyl aminopeptidase from *Lactococcus lactis* spp. *lactis*. *Ultrason. Sonochem.* **29**, 163–171 (2016).
190. L. Heydari, R. K. Kermanshahi, S. Gharavi, Z. Moosavi-Nejad, Characterization of the recombinant PepX peptidase from *Lactobacillus fermentum* and its effect on gliadin protein hydrolysis in vitro. *Biologia* **78**, 565–577 (2023).
191. J. Goldstein, A. Banbula, T. Kordula, J. Mayo, J. Travis, Novel extracellular x-prolyl dipeptidyl-peptidase (DPP) from *Streptococcus gordonii* FSS2: An emerging subfamily of viridans streptococcal x-prolyl DPPs. *Infect. Immun.* **69**, 5494–5501 (2001).
192. P. Glaser, C. Rusniok, C. Buchrieser, F. Chevalier, L. Frangeul, T. Msadek, M. Zouine, E. Couvé, L. Lalioui, C. Poyart, Genome sequence of *Streptococcus agalactiae*, a pathogen causing invasive neonatal disease. *Mol. Microbiol.* **45**, 1499–1513 (2002).

193. A. De, A. Pompilio, J. Francis, I. C. Sutcliffe, G. W. Black, G. Lupidi, D. Petrelli, L. A. Vitali, Antidiabetic "gliptins" affect biofilm formation by *Streptococcus mutans*. *Microbiol. Res.* **209**, 79–85 (2018).
194. S. M. Rudman, S. Greenblum, R. C. Hughes, S. Rajpurohit, O. Kiratli, D. B. Lowder, S. G. Lemmon, D. A. Petrov, J. M. Chaston, P. Schmidt, Microbiome composition shapes rapid genomic adaptation of *Drosophila melanogaster*. *Proc. Natl. Acad. Sci. U.S.A.* **116**, 20025–20032 (2019).
195. M. A. Hanson, L. Grollmus, B. Lemaitre, Ecology-relevant bacteria drive the evolution of host antimicrobial peptides in *Drosophila*. *Science* **381**, eadg5725 (2023).
196. X. Didelot, A. S. Walker, T. E. Peto, D. W. Crook, D. J. Wilson, Within-host evolution of bacterial pathogens. *Nat. Rev. Microbiol.* **14**, 150–162 (2016).
197. A. Caminero, M. Guzman, J. Libertucci, A. E. Lomax, The emerging roles of bacterial proteases in intestinal diseases. *Gut Microbes* **15**, 2181922 (2023).
198. J. Jumper, R. Evans, A. Pritzel, T. Green, M. Figurnov, O. Ronneberger, K. Tunyasuvunakool, R. Bates, A. Žídek, A. Potapenko, Highly accurate protein structure prediction with AlphaFold. *Nature* **596**, 583–589 (2021).
199. D. S. Goodsell, M. F. Sanner, A. J. Olson, S. Forli, The AutoDock suite at 30. *Protein Sci.* **30**, 31–43 (2021).
200. The PyMOL Molecular Graphics System, Version 1.2r3pre, Schrödinger, LLC; https://www.pymol.org/.
201. J. Yang, D. Sun-Waterhouse, C. Cui, K. Dong, M. Zhao, γ-glu-met synthesised using a bacterial glutaminase as a potential inhibitor of dipeptidyl peptidase IV. *Int. J. Food Sci. Technol.* **53**, 1166–1175 (2018).
202. R. Bhatia, S. Singh, R. Maurya, S. K. Bhadada, M. Bishnoi, K. Chopra, S. R. Joshi, K. K. Kondepudi, In vitro characterization of lactic acid bacterial strains isolated from fermented foods with anti-inflammatory and dipeptidyl peptidase-IV inhibition potential. *Braz. J. Microbiol.* **54**, 293–309 (2023).
203. R. Fontoura, D. J. Daroit, A. P. Correa, S. M. Meira, M. Mosquera, A. Brandelli, Production of feather hydrolysates with antioxidant, angiotensin-I converting enzyme- and dipeptidyl peptidase-IV-inhibitory activities. *N. Biotechnol.* **31**, 506–513 (2014).
204. J. Yang, Y. Lee, Y. Kim, S. Lee, S. Ryu, S. Fukuda, K. Hase, C. Yang, H. Lim, M. Kim, Gut commensal *Bacteroides acidifaciens* prevents obesity and improves insulin sensitivity in mice. *Mucosal Immunol.* **10**, 104–116 (2017).
205. T. Akiyama, M. Abe, S. Harada, F. Kojima, R. Sawa, Y. Takahashi, H. Naganawa, Y. Homma, M. Hamada, A. Yamaguchi, Sulphostin, a potent inhibitor for dipeptidyl peptidase IV from *Streptomyces* sp. MK251-43F3. *J. Antibiot.* **54**, 744–746 (2001).
206. Z. Zeng, J. Y. Luo, F. L. Zuo, R. Yu, Y. Zhang, H. Q. Ma, S. W. Chen, Bifidobacteria possess inhibitory activity against dipeptidyl peptidase-IV. *Lett. Appl. Microbiol.* **62**, 250–255 (2016).
207. K. Imamura, Y. Tsuyama, T. Hirata, Identification and characterization of a novel fermented substance produced by edible *Aspergillus oryzae* AO-1 that inhibits DPP-IV activity. *J. Biosci. Bioeng.* **111**, 37–40 (2011).
208. Y. Takei, "Appetite-regulating peptides" in *Handbook of Hormones* (Elsevier, 2021), pp. 125–127.
209. C. Hölscher, Glucagon-like peptide 1 and glucose-dependent insulinotropic peptide hormones and novel receptor agonists protect synapses in Alzheimer's and Parkinson's diseases. *Front. Synaptic Neurosci.* **14**, 955258 (2022).
210. H. Ando, K. Ukena, S. Nagata *Handbook of Hormones: Comparative Endocrinology for Basic and Clinical Research* (Academic Press, 2021).
211. N. K. Leeuwendaal, J. F. Cryan, H. Schellekens, Gut peptides and the microbiome: Focus on ghrelin. *Curr. Opin. Endocrinol. Diabetes Obes.* **28**, 243–252 (2021).
212. T. B. Usdin, E. Mezey, D. C. Button, M. J. Brownstein, T. I. Bonner, Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology* **133**, 2861–2870 (1993).
213. E. Faivre, C. Hölscher, Neuroprotective effects of D-Ala2GIP on Alzheimer's disease biomarkers in an APP/PS1 mouse model. *Alzheimers Res. Ther.* **5**, 20 (2013).
214. C. P. Figueiredo, F. A. Pamplona, T. L. Mazzuco, A. S. Aguiar Jr., R. Walz, R. D. S. Prediger, Role of the glucose-dependent insulinotropic polypeptide and its receptor in the central nervous system: Therapeutic potential in neurological diseases. *Behav. Pharmacol.* **21**, 394–408 (2010).
215. C. Ji, G.-F. Xue, G. Li, D. Li, C. Hölscher, Neuroprotective effects of glucose-dependent insulinotropic polypeptide in Alzheimer's disease. *Rev. Neurosci.* **27**, 61–70 (2016).
216. E. Faivre, A. Hamilton, C. Hölscher, Effects of acute and chronic administration of GIP analogues on cognition, synaptic plasticity and neurogenesis in mice. *Eur. J. Pharmacol.* **674**, 294–306 (2012).
217. E. Faivre, V. A. Gault, B. Thorens, C. Hölscher, Glucose-dependent insulinotropic polypeptide receptor knockout mice are impaired in learning, synaptic plasticity, and neurogenesis. *J. Neurophysiol.* **105**, 1574–1580 (2011).
218. D. J. Drucker, Glucagon-like peptide 2. *J. Clin. Endocrinol. Metab.* **86**, 1759–1764 (2001).
219. D. J. Drucker, Biological actions and therapeutic potential of the glucagon-like peptides. *Gastroenterology* **122**, 531–544 (2002).
220. S. Sasaki-Hamada, M. Ikeda, J.-I. Oka, Glucagon-like peptide-2 rescues memory impairments and neuropathological changes in a mouse model of dementia induced by the intracerebroventricular administration of streptozotocin. *Sci. Rep.* **9**, 13723 (2019).
221. A. Amato, F. Mulè, Protective potential of glucagon like peptide 2 (GLP-2) against the neurodegeneration. *Neural Regen. Res.* **14**, 1901 (2019).
222. M. C. Simon, K. Strassburger, B. Nowotny, H. Kolb, P. Nowotny, V. Burkart, F. Zivehe, J. H. Hwang, P. Stehle, G. Pacini, B. Hartmann, J. J. Holst, C. MacKenzie, L. B. Bindels, I. Martinez, J. Walter, B. Henrich, N. C. Schloot, M. Roden, Intake of *Lactobacillus reuteri* improves incretin and insulin secretion in glucose-tolerant humans: A proof of concept. *Diabetes Care* **38**, 1827–1834 (2015).
223. A. M. Lynch, N. Pathak, Y. E. Flatt, V. A. Gault, F. P. O'Harte, N. Irwin, P. R. Flatt, Comparison of stability, cellular, glucose-lowering and appetite supressing effects of oxyntomodulin analogues modified at the N-terminus. *Eur. J. Pharmacol.* **743**, 69–78 (2014).
224. B. T. Schjoldager, F. G. Baldissera, P. E. Mortensen, J. J. Holst, J. Christiansen, Oxyntomodulin: A potential hormone from the distal gut. *Pharmacokinetics* and effects on gastric acid and insulin secretion in man. *Eur. J. Clin. Invest.* **18**, 499–503 (1988).
225. C. Moss, W. S. Dhillo, G. Frost, M. Hickson, Gastrointestinal hormones: The regulation of appetite and the anorexia of ageing. *J. Hum. Nutr. Diet.* **25**, 3–15 (2012).
226. R. T. Long, W. S. Zeng, L. Y. Chen, J. Guo, Y. Z. Lin, Q. S. Huang, S. Q. Luo, *Bifidobacterium* as an oral delivery carrier of oxyntomodulin for obesity therapy: Inhibitory effects on food intake and body weight in overweight mice. *Int. J. Obes.* **34**, 712–719 (2010).
227. A. Balasubramaniam, Clinical potentials of neuropeptide Y family of hormones. *Am. J. Surg.* **183**, 430–434 (2002).
228. X. Pedragosa-Badia, J. Stichel, A. G. Beck-Sickinger, Neuropeptide Y receptors: How to get subtype selectivity. *Front. Endocrinol.* **4**, 5 (2013).
229. J. Breton, P. Tirelle, S. Hasanat, A. Pernot, C. L'Huillier, J. C. do Rego, P. Dechelotte, M. Coeffier, L. B. Bindels, D. Ribet, Gut microbiota alteration in a mouse model of *Anorexia Nervosa*. *Clin. Nutr.* **40**, 181–189 (2021).
230. L.-J. Sun, J.-N. Li, Y.-Z. Nie, Gut hormones in microbiota-gut-brain cross-talk. *Chin. Med. J.* **133**, 826–833 (2020).
231. D. Verma, J. Wood, G. Lach, H. Herzog, G. Sperk, R. Tasan, Hunger promotes fear extinction by activation of an amygdala microcircuit. *Neuropsychopharmacology* **41**, 431–439 (2016).
232. U. Stadlbauer, W. Langhans, U. Meyer, Administration of the Y2 receptor agonist PYY3-36 in mice induces multiple behavioral changes relevant to schizophrenia. *Neuropsychopharmacology* **38**, 2446–2455 (2013).
233. A. De Silva, S. R. Bloom, Gut hormones and appetite control: A focus on PYY and GLP-1 as therapeutic targets in obesity. *Gut Liver* **6**, 10 (2012).
234. Y. Wang, Y. Wu, J. Sailike, X. Sun, N. Abuduwaili, H. Tuoliuhan, M. Yusufu, X. H. Nabi, Fourteen composite probiotics alleviate type 2 diabetes through modulating gut microbiota and modifying M1/M2 phenotype macrophage in db/db mice. *Pharmacol. Res.* **161**, 105150 (2020).
235. H. P. M. van der Kleij, J. Bienenstock, "Significance of sensory neuropeptides and the immune response" in *Psychoneuroimmunology* (Academic Press, 2007).
236. M. Patel, S. V. Subas, M. R. Ghani, V. Busa, A. Dardeir, S. Marudhai, I. Cancarevic, Role of substance P in the pathophysiology of inflammatory bowel disease and its correlation with the degree of inflammation. *Cureus* **12**, e11027 (2020).
237. L. Wang, S. Ahmad, I. F. Benter, A. Chow, S. Mizutani, P. E. Ward, Differential processing of substance P and neurokinin A by plasma dipeptidyl (amino) peptidase IV, aminopeptidase M and angiotensin converting enzyme. *Peptides* **12**, 1357–1364 (1991).
238. J. M. Yano, K. Yu, G. P. Donaldson, G. G. Shastri, P. Ann, L. Ma, C. R. Nagler, R. F. Ismagilov, S. K. Mazmanian, E. Y. Hsiao, Indigenous bacteria from the gut microbiota regulate host serotonin biosynthesis. *Cell* **161**, 264–276 (2015).
239. V. Appay, S. L. Rowland-Jones, RANTES: A versatile and controversial chemokine. *Trends Immunol.* **22**, 83–87 (2001).
240. R. Mentlein, Dipeptidyl-peptidase IV (CD26)-role in the inactivation of regulatory peptides. *Regul. Pept.* **85**, 9–24 (1999).
241. D. Tripathy, L. Thirumangalakudi, P. Grammas, RANTES upregulation in the Alzheimer's disease brain: A possible neuroprotective role. *Neurobiol. Aging* **31**, 8–16 (2010).
242. A. Valerio, M. Ferrario, F. O. Martinez, M. Locati, V. Ghisi, L. G. Bresciani, A. Mantovani, P. Spano, Gene expression profile activated by the chemokine CCL5/RANTES in human neuronal cells. *J. Neurosci. Res.* **78**, 371–382 (2004).
243. M. Mazhar, Y. Zhu, L. Qin, The interplay of dietary fibers and intestinal microbiota affects type 2 diabetes by generating short-chain fatty acids. *Foods* **12**, 1023 (2023).
244. S. Singh, R. Bhatia, P. Khare, S. Sharma, S. Rajarammohan, M. Bishnoi, S. K. Bhadada, S. S. Sharma, J. Kaur, K. K. Kondepudi, Anti-inflammatory *Bifidobacterium* strains prevent dextran sodium sulfate induced colitis and associated gut microbial dysbiosis in mice. *Sci. Rep.* **10**, 18597 (2020).
245. W. Wang, B. K. Choi, W. Li, Z. Lao, A. Y. Lee, S. C. Souza, N. A. Yates, T. Kowalski, A. Pocai, L. H. Cohen, Quantification of intact and truncated stromal cell-derived factor-1α in

circulation by immunoaffinity enrichment and tandem mass spectrometry. *J. Am. Soc. Mass Spectrom.* **25**, 614–625 (2014).
246. M. Li, J. S. Hale, J. N. Rich, R. M. Ransohoff, J. D. Lathia, Chemokine CXCL12 in neurodegenerative diseases: An SOS signal for stem cell-based repair. *Trends Neurosci.* **35**, 619–628 (2012).
247. Q. Wang, Y. Xu, J.-C. Chen, Y.-Y. Qin, M. Liu, Y. Liu, M.-J. Xie, Z.-Y. Yu, Z. Zhu, W. Wang, Stromal cell-derived factor 1α decreases β-amyloid deposition in Alzheimer's disease mouse model. *Brain Res.* **1459**, 15–26 (2012).
248. C.-C. Wu, I.-F. Wang, P.-M. Chiang, L.-C. Wang, C.-K. J. Shen, K.-J. Tsai, G-CSF-mobilized bone marrow mesenchymal stem cells replenish neural lineages in Alzheimer's disease mice via CXCR4/SDF-1 chemotaxis. *Mol. Neurobiol.* **54**, 6198–6212 (2017).
249. B. A. Teicher, S. P. Fricker, CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clin. Cancer Res.* **16**, 2927–2931 (2010).
250. J. Lipfert, V. Ödemis, D. C. Wagner, J. Boltze, J. Engele, CXCR 4 and CXCR 7 form a functional receptor unit for SDF-1/CXCL 12 in primary rodent microglia. *Neuropathol. Appl. Neurobiol.* **39**, 667–680 (2013).
251. K. W. Christopherson, G. Hangoc, C. R. Mantel, H. E. Broxmeyer, Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* **305**, 1000–1003 (2004).
252. Y. Heshiki, R. Vazquez-Uribe, J. Li, Y. Ni, S. Quainoo, L. Imamovic, J. Li, M. Sorensen, B. K. C. Chow, G. J. Weiss, A. Xu, M. O. A. Sommer, G. Panagiotou, Predictable modulation of cancer treatment outcomes by the gut microbiota. *Microbiome* **8**, 28 (2020).
253. S. Jia, J. Li, B. Yu, M. Li, B. Cui, Improvement of myocardial injury and gut microbiota disturbance in type 2 diabetic mice by inulin with various degrees of polymerization. *Food Biosci.* **51**, 102318 (2023).
254. C. Marchetti, A. Di Carlo, F. Facchiano, C. Senatore, R. De Cristofaro, A. Luzi, M. Federici, M. Romani, M. Napolitano, M. C. Capogrossi, A. Germani, High mobility group box 1 is a novel substrate of dipeptidyl peptidase-IV. *Diabetologia* **55**, 236–244 (2012).
255. X. Yang, X. Cheng, Y. Tang, X. Qiu, Z. Wang, G. Fu, J. Wu, H. Kang, J. Wang, H. Wang, F. Chen, X. Xiao, T. R. Billiar, B. Lu, The role of type 1 interferons in coagulation induced by gram-negative bacteria. *Blood* **135**, 1087–1100 (2020).
256. L. L. Mantell, V. Patel, R. Sitapara, HMGB1 mediates hyperoxia-compromised host defense to clear bacteria in pseudomonas aeruginosa pneumonia. *Free Radic. Biol. Med.* **53**, S161 (2012).
257. A. Sato, S. Suzuki, S. Watanabe, T. Shimizu, Y. Nakamura, T. Misaka, T. Yokokawa, T. Shishido, S. I. Saitoh, T. Ishida, I. Kubota, Y. Takeishi, DPP4 inhibition ameliorates cardiac function by blocking the cleavage of HMGB1 in diabetic mice after myocardial infarction. *Int. Heart J.* **58**, 778–786 (2017).
258. L. R. Potter, A. R. Yoder, D. R. Flora, L. K. Antos, D. M. Dickey, "Natriuretic peptides: Their structures, receptors, physiologic functions and therapeutic applications" in *cGMP: Generators, Effectors and Therapeutic Implications*, H. H. H. W. Schmidt, F. Hofmann, J.-P. Stasch, Eds. (Springer, 2009), pp. 341–366.
259. L. Mu, Z. Wang, J. Ren, X. Xiong, Z. Jin, X. Liu, Impact of DPP-4 inhibitors on plasma levels of BNP and NT-pro-BNP in type 2 diabetes mellitus. *Diabetol. Metab. Syndr.* **14**, 30 (2022).
260. R. Roesler, P. Kent, T. Luft, G. Schwartsmann, Z. Merali, Gastrin-releasing peptide receptor signaling in the integration of stress and memory. *Neurobiol. Learn. Mem.* **112**, 44–52 (2014).
261. H. L. Sun, Q. Y. Ma, H. G. Bian, X. M. Meng, J. Jin, Novel insight on GRP/GRPR axis in diseases. *Biomed. Pharmacother.* **161**, 114497 (2023).
262. D. Rat, U. Schmitt, F. Tippmann, I. Dewachter, C. Theunis, E. Wieczerzak, R. Postina, F. van Leuven, F. Fahrenholz, E. Kojro, Neuropeptide pituitary adenylate cyclase-activating polypeptide (PACAP) slows down Alzheimer's disease-like pathology in amyloid precursor protein-transgenic mice. *FASEB J.* **25**, 3208–3218 (2011).
263. M. M. Heimesaat, G. Reifenberger, V. Vicena, A. Illes, G. Horvath, A. Tamas, B. D. Fulop, S. Bereswill, D. Reglodi, Intestinal microbiota changes in mice lacking pituitary adenylate cyclase activating polypeptide (PACAP)—Bifidobacteria make the difference. *Eur. J. Microbiol. Immunol.* **7**, 187–199 (2017).
264. M. Bains, C. Laney, A. E. Wolfe, M. Orr, J. A. Waschek, A. C. Ericsson, G. P. Dorsam, Vasoactive intestinal peptide deficiency is associated with altered gut microbiota communities in male and female C57BL/6 mice. *Front. Microbiol.* **10**, 2689 (2019).

Acknowledgments

Funding: The Microbiome Core is funded, in part, by the Center for Gastrointestinal Biology and Disease (CGIBD P30 DK034987) and the UNC Nutrition Obesity Research Center (N ORC P30DK056350). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication. **Author contributions:** M.R.A.: Writing—original draft, conceptualization, writing—review and editing, methodology, data curation, project administration, and visualization. J.R.: Writing—original draft, conceptualization, writing—review and editing, methodology, resources, data curation, validation, formal analysis, software, and visualization. Y.H.: Writing—original draft, conceptualization, investigation, writing—review and editing, resources, and data curation. J.G.: Conceptualization, writing—review and editing, methodology, and resources. M.A.A.-P.: Writing—original draft, conceptualization, investigation, writing—review and editing, resources, funding acquisition, supervision, and project administration. **Competing interests:** The authors declare that they have no competing interests.

Submitted 19 August 2024
Accepted 26 February 2025
Published 2 April 2025
10.1126/sciadv.ads5721