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Review

Advanced microbiome therapeutics as a novel modality for oral delivery of peptides to manage metabolic diseases

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The rising prevalence of metabolic diseases calls for innovative treatments. Peptide-based drugs have transformed the management of conditions such as obesity and type 2 diabetes. Yet, challenges persist in oral delivery of these peptides. This review explores the potential of 'advanced microbiome therapeutics' (AMTs), which involve engineered microbes for delivery of peptides *in situ*, thereby enhancing their bioavailability. Preclinical work on AMTs has shown promise in treating animal models of metabolic diseases, including obesity, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease. Outstanding challenges toward realizing the potential of AMTs involve improving peptide expression, ensuring predictable colonization control, enhancing stability, and managing safety and biocontainment concerns. Still, AMTs have potential for revolutionizing the treatment of metabolic diseases, potentially offering dynamic and personalized novel therapeutic approaches.

Highlights

Proteins and peptides derived from hormones hold huge potential for preventing and treating metabolic diseases.

Challenges in administering peptide-based medicines include their short-lived nature, poor bioavailability, and reliance on methods such as injection, which might discourage people from medication adherence.

Advanced microbiome therapeutics (AMTs) involving engineered microbes have emerged as a potential solution for *in situ* therapeutic production and delivery of peptides and proteins, supported by preclinical data in animal models of metabolic disorders, such as obesity, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease (MASLD).

Current challenges for AMT development include colonization control, safety, and biocontainment.

AMTs not only may address practical challenges but also may have socioeconomic impact by making therapeutic peptides more affordable.

The potential of AMTs could transform the treatment of metabolic disorders, offering more dynamic and personalized therapeutic approaches.

Prevalence and current treatment options for metabolic diseases

The prevalence of metabolic diseases has increased in the past century, with conditions such as obesity, type 2 diabetes, and metabolic dysfunction-associated steatotic liver disease (MASLD) becoming more widespread [1–3]. These diseases are associated with an increased (cardiovascular) morbidity and mortality risk [4]. Indeed, over one billion people are now considered as living with obesity, as reported by the World Health Organization (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). While genetics predispose certain individuals to these diseases, it is difficult to pinpoint a singular cause for their increased incidence [5,6]. There is evidence that multiple external factors play important roles, including shifts in dietary patterns toward more energy-dense processed foods [7], sedentary lifestyles [8], and socioeconomic factors [9].

Imbalances in pathways regulating glucose and fat metabolism, nutrient absorption, satiety, and hormonal action all contribute to body weight gain and elevate the risk of obesity, type 2 diabetes, or MASLD [10–12]. Exogenous insulin replacement therapy, a century-old and widely accepted treatment for type 2 diabetes, remains a safe and effective option despite the associated weight gain caused by insulin [13].

In addressing these metabolic diseases, a class of peptide-based therapeutics has emerged, holding significant potential with numerous molecules. One notable success within this category involves drugs that mimic or modulate the gastrointestinal hormone glucagon-like peptide-1 (GLP-1). Exenatide (Byetta), the first GLP-1 drug, approved in 2005 for managing type 2 diabetes, is a GLP-1 receptor agonist derived from the saliva of the Gila monster, with 50% homology to human GLP-1 but an extended half-life [14]. Subsequent improvement in peptide stability resulted

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in liraglutide (Victoza), approved in 2010, modeled on human GLP-1 with a significantly prolonged half-life [15], gaining FDA approval in 2014 for obesity treatment. A more effective version of these drugs followed: semaglutide [16] (injectable form Ozempic/Wegovy, and in oral form Rybelsus, which received FDA approval in 2016 and 2019, respectively). In 2023, a noteworthy addition to this therapeutic landscape emerged with the approval by the FDA of tirzepatide (Zepbound), a dual glucose-dependent insulinotropic polypeptide (GIP) and GLP-1 receptor agonist.

These gut peptide-based therapeutics have markedly improved the quality of life for patients with diabetes and patients with obesity. Clinical trials exploring their potential in treating MASLD are underway. However, GLP-1 receptor agonists represent just the 'tip of the iceberg' of potential peptide-based drugs [17] (Figure 1). Most of these mammalian-derived molecules are small peptides, which are usually short-lived [18]. Because of their labile nature and poor oral bioavailability, peptide-based medicines are often administered by intravenous, intramuscular, or subcutaneous injection [19].

Given the potential of these peptide-based therapeutics, alternative strategies for administration are urgently needed. While oral administration is the preferred route for medicines, it poses a set of challenges for peptide-based drugs. In this review, we discuss a novel approach to deliver peptide-based medicines orally using engineered probiotic microbes. This approach is referred to as AMTs [20–22], and the following text focuses on the preclinical experience of AMTs and their potential application in treating metabolic diseases, along with the challenges to overcome.

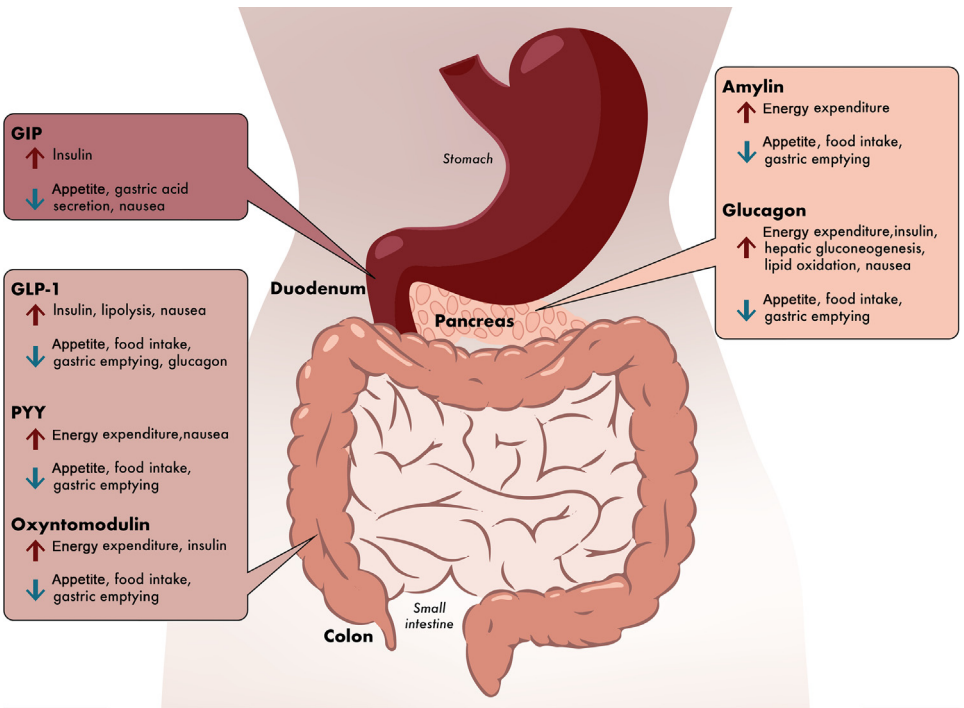

Figure 1. Therapeutic potential of gastrointestinal peptides. This figure illustrates peptides that originated from the gastrointestinal tract, showcasing their therapeutic potential. Abbreviations: GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; PYY, peptide YY.

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Limitations of current peptide-based treatments

Peptide-based medicines, whether injectables or orals, come with inherent limitations. Injectable peptides, typically administered through subcutaneous or intravenous routes, pose challenges related to patient acceptance and compliance, particularly for self-administration [23,24]. Oral administration of peptides offers a more patient-friendly alternative, avoiding the challenges associated with injections, such as pain and the need for specialized devices. However, oral peptide delivery faces numerous challenges due to the need to overcome various structural and functional barriers. A brief overview is provided here for context; a comprehensive review on this subject is available elsewhere [19,25].

Despite their small size (2–50 amino acids [26]), peptides often exhibit low absorption across the intestinal barrier. Furthermore, given that peptides are composed of amino acids, they generally have poor stability in the gastrointestinal environment (e.g., degradation by stomach acid). The primary challenges in oral peptide delivery are summarized in Box 1.

The challenges for oral delivery of peptides are significant because an essential function of the digestive system is to break down ingested proteins into easily absorbed smaller molecules. Nonetheless, there are multiple strategies that have been explored to overcome these barriers, such as cell-penetrating peptides [27,28], fusion peptides [29], chemical modifications to increase stability [15,30], permeation enhancers [31], inhibitors of gut enzymes [32], and nanotechnology-based devices [33]. However, no robust framework or general solution for oral delivery of peptides exists. Each peptide has its own challenge, representing a significant barrier to applying peptide-based medicines.

Recent advances in synthetic biology have enabled the precise engineering of probiotics and commensal gut microorganisms to continuously produce therapeutic peptides directly in the gastrointestinal tract. Therefore, this is an intriguing alternative that may circumvent some of the challenges faced by traditional oral delivery of therapeutic peptides.

AMTs in metabolic diseases

Synthetic biology has revolutionized medical biotechnology by focusing on designing and constructing biological components and systems for the controlled production of small molecules or proteins in laboratory settings. However, a significant and promising application area within

Box 1. Challenges in oral peptide delivery

Rapid degradation

The gastrointestinal tract specializes in digestion, rendering oral peptides susceptible to enzymatic and environmental degradation, particularly in the stomach and small intestine. Proteolytic enzymes in saliva, the stomach, and intestines can break down peptides into smaller fragments, reducing their bioavailability. Peptides are particularly susceptible to degradation by gastric enzymes such as pepsin and pancreatic enzymes such as trypsin and chymotrypsin [108]. In addition, peptides may be sensitive to the acidic environment of the stomach, leading to degradation before they reach the absorption site in the small intestine. In the gastrointestinal tract, the enzymatic environment within the lumen, including the enzymatic activity of gut microbes [109], and pH can impact the stability of certain peptides. This rapid degradation reduces their bioavailability and efficacy when administered orally, requiring frequent administration to maintain therapeutic levels, which can be a logistical challenge for patients with chronic conditions.

Poor absorption

Peptides often exhibit low absorption across the gastrointestinal tract. The mucus barrier within the gastrointestinal epithelium poses an initial physical hurdle, impeding effective penetration and absorption. Moreover, biological membranes and tight junctions between epithelial cells restrict paracellular transport, whereas the transcellular route through absorptive enterocytes presents challenges due to potential lysosomal degradation of foreign intracellular proteins [110]. These biological barriers limit peptide absorption into the bloodstream.

medicinal synthetic biology has been somewhat overlooked – the development of engineered microbes as delivery systems for *in situ* therapeutic production [34,35]. This is particularly relevant for peptide-based drugs, as challenges associated with oral peptide administration could potentially be overcome through innovative strategies in engineering commensal gut bacteria and yeast, termed AMTs.

The use of AMTs in metabolic diseases holds significant promise because of their potential to address specific challenges associated with administering oral peptides for treatment. Promising preclinical results in this field are highlighted in Table 1. These examples are discussed in the next section in the context of different metabolic diseases, emphasizing the potential of AMTs to contribute significantly to their treatment.

AMTs for treatment of obesity

Obesity is characterized by the accumulation of excess body fat, which is associated with substantial comorbidities and responsible for 2.8 million deaths each year (https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight). Addressing obesity often involves lifestyle modifications, such as adopting a healthy diet, increasing physical activity, and behavior changes. However, medical interventions, such as medications or surgery, may be recommended, especially when obesity poses significant health risks. GLP-1 receptor agonists have proved particularly effective in treating obesity [36]. Despite its beneficial properties, native GLP-1 undergoes rapid degradation [37], requiring the development of strategies to enhance its efficacy and bioavailability for oral delivery as a therapeutic agent.

Pioneering studies have successfully demonstrated the positive effect of AMTs engineered to produce GLP-1 receptor agonist in mouse models of obesity [22,38]. Overall, the studies showcased the efficacy of two engineered probiotic strains, *Escherichia coli* Nissle and *Saccharomyces boulardii*, in delivering GLP-1 and exendin-4 (a GLP-1 analogue). Notably, these interventions resulted in a significant reduction in weight gain and food intake in mice subjected to a high-fat diet.

Interestingly, one of the studies employed a combination of *in vitro* and animal models to assess the effectiveness of GLP-1– and exendin-4-producing AMT strains in stimulating insulin secretion from isolated pancreatic islets [22]. The authors noted an enhanced effect by the exendin-4 strain, attributed to the longer half-life of the peptide, which led to further testing the strain in animal models.

Furthermore, in another study, researchers investigated the peptide oxyntomodulin, an appetite-suppressing peptide with therapeutic potential [39]. In this work, an AMT was developed for *in situ* delivery of the peptide by an engineered *Bifidobacterium longum*. A significant reduction was obtained in food intake, body weight, and blood triglyceride levels in mice with obesity. Additionally, a decrease in the hunger-promoting hormone ghrelin was observed. Oxyntomodulin has a dual mechanism, acting on the central nervous system (similar to GLP-1) and inhibiting ghrelin release, positioning it as a promising therapeutic candidate for obesity.

AMTs for treatment of type 2 diabetes

Type 2 diabetes is a chronic metabolic disease characterized by high levels of blood glucose resulting from the body's inability to effectively use insulin or insufficient production of insulin. Factors contributing to the development of type 2 diabetes include a poor diet, lack of physical activity, and genetic predisposition [40]. The management of diabetes involves maintaining blood sugar levels within a target range. Therefore, GLP-1 receptor agonists were initially designed as a therapeutic intervention for type 2 diabetes [41,42]. Unlike insulin, GLP-1 operates through glucose-dependent mechanisms, reducing the risk of hypoglycemia.

Table 1. Overview of AMT preclinical studies to treat metabolic diseases[a]

Disease	Chassis	Genetic modification	Preclinical model used for validation	Summary of outcomes	Refs
Obesity	*Bifidobacterium longum*	Oxyntomodulin	Overweight female BALB/c mice fed a high-fat diet	• Food intake, body weight, and blood triglyceride levels of overweight treated mice were significantly reduced compared with control groups. • The levels of oxyntomodulin in the intestinal contents of the treated group were significantly increased compared with those in the control groups. • Plasma levels of the hunger-promoting hormone ghrelin in the treated group were significantly reduced.	[39]
Obesity	*Escherichia coli* Nissle	Modified GLP-1	Lean SPF mice fed a high-fat diet	• Treatment led to a significant decrease in body weight gain and reduced food intake in mice fed a high-fat diet. • Adipose (fat) pad and liver weights decreased with treatment. • Improved glucose tolerance observed with treatment. • Treatment improved hepatic histology and biochemistry, suggesting potential protection in obese mice's livers.	[38]
Obesity	*Saccharomyces boulardii*	Exendin-4 and GLP-1	*Ex vivo* (isolated mice pancreatic islets) and lean male C57BL/6 mice fed a high-fat diet	• Treatment effectively stimulated insulin secretion from isolated pancreatic islets. • Treated mice exhibited stronger suppression of food intake and body weight gain during cold exposure compared with room temperature conditions.	[22]
Diabetes	*Lactococcus lactis*	GLP-1	*In vitro* assay using HIT-T15 master cell line to study insulin secretion, an *in vitro* assay using MDCK cell monolayers to assess the transport of GLP-1 and ZDF rats	• Engineered strain demonstrated significant insulinotropic activity on HIT-T15 cells *in vitro*. • Enhanced bioavailability in MDCK monolayer transport assay (eight times higher than GLP-1 in free solution). • Significant decrease (10%–20%) in blood glucose levels during 2–11 h postdosing in ZDF rats. • Improved insulin response.	[45]
Diabetes	*Lactobacillus gasseri*	GLP-1	IEC-6 rat intestinal stem cells to study GLP-1R in mediating reprogramming and female Wistar rats treated with streptozotocin	• Diabetic rats fed the engineered strain showed increased insulin levels and improved glucose tolerance. • Rats treated with the engineered strain developed insulin-producing cells in the upper intestine, replacing 25%–33% of insulin capacity.	[43]
Diabetes	*L. lactis*	GLP-1	*Ex vivo* (isolated mice pancreatic islets) and lean male C57BL/6 mice fed chow and high-fat diet	• Engineered strain effectively stimulated insulin secretion from isolated pancreatic islets. • Treatment increased circulating GLP-1 levels in both chow and high-fat diet-fed mice. • Engineered strain improved glucose tolerance in both chow and high-fat diet-fed mice.	[44]
Diabetes	*Lactobacillus paracasei*	5xGLP-1	Pancreatic islet isolation from Wistar rats and the use of the HIT-T15 cell line were employed to test the insulinotropic effect of the strains. Male Goto-Kakizaki rats, serving as an animal model for type 2 diabetes, were used in the study.	• *In vitro*, the pentameric GLP-1 was converted into monomeric GLP-1 and stimulated insulin secretion from HIT-T15 cells. • Intraperitoneal administration of recombinant 5xGLP-1 alone resulted in significantly improved glycemic control. • Feeding the engineered strain for 14 days resulted in a significant decrease in blood glucose levels compared with the control.	[46]
MASLD	*E. coli* Nissle	Aldafermin	C57BL/6J male mice fed an American lifestyle-induced obesity syndrome diet	• Mice treated with the engineered strain, along with dietary changes, resulted in reduced body weight, liver steatosis (as assessed by histology), decreased plasma aspartate aminotransferase levels, and reduced cholesterol levels (including total cholesterol, HDL-C, and LDL-C). • Transcriptomic and metabolomic analyses provided insights into the underlying molecular pathways, indicating improvements in amino acid and lipid metabolism, oxidative stress, and insulin resistance.	[55]

[a]Abbreviations: HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MDCK, Madin-Darby canine kidney; SPF, specific pathogen-free; ZDF, Zucker Diabetic Fatty.

Several AMTs have been evaluated for delivering GLP-1 in animal models of diabetes [43–46], predominantly using probiotic lactic acid bacteria such as *Lactococcus lactis, Lactobacillus gasseri*, and *Lactobacillus paracasei*. These AMTs have undergone extensive assessment in both *in vitro* and animal models involving mice and rats, demonstrating improvements in insulin secretion and enhanced glucose tolerance.

A particularly intriguing observation in one of the studies highlights that GLP-1, when delivered via an AMT directly into the gastrointestinal tract, stimulates the transformation of rat intestinal cells into insulin-secreting cells [43]. This revelation opens an intriguing avenue for exploring the use of AMTs to influence intestinal cell differentiation.

AMTs for treatment of MASLD

MASLD is an umbrella term used for chronic liver diseases associated with hepatocellular lipid accumulation, impaired hormonal signaling, and upregulated proinflammatory processes. It affects 25% of the world's population [4], but the pathogenesis and progression of MASLD are poorly understood. Yet, genetic factors, physical activity, diet, and the gut microbiome play important roles [47–49].

In addition to these factors, endocrine imbalances and other metabolic diseases, such as obesity, are frequently linked to MASLD. Hormones such as fibroblast growth factor-19 (FGF-19) [50,51], insulin-like growth factor-1 (IGF-1) [52], and glucagon-like peptide-1 (GLP-1) [53,54] are reported to be downregulated during MASLD. FGF-19 and GLP-1 play important roles in regulating hepatic lipid metabolism. In that manner, restoring hormonal homeostasis emerges as a potential target for MASLD treatment, as it can interrupt the cascade of metabolic events contributing to disease progression.

Despite various attempts to develop specific drugs currently there is no approved treatment for MASLD, and management relies on lifestyle modifications focusing on weight loss through diet and exercise. However, the prevalence of MASLD is rapidly increasing, necessitating more effective treatment strategies.

One instance of an AMT for MASLD involved the engineering of an *E.coli* Nissle strain to produce aldafermin, an FGF-19 analogue, which was evaluated as a potential treatment in a mouse model for MASLD [55]. Mice treated with the engineered strain, combined with dietary changes, exhibited reduced body weight, liver steatosis, decreased plasma aspartate aminotransferase (AST) levels, and reduced cholesterol levels [including total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C)]. Furthermore, transcriptomic and metabolomic analyses provided insights into underlying molecular pathways, suggesting improvements in amino acid and lipid metabolism, oxidative stress, and insulin resistance.

Despite the considerable potential of hormone peptides discussed in this section, the journey toward developing pharmaceutical formulations of them is a time- and resource-intensive process. The GLP-1 narrative, spanning from its discovery in 1980 to its current status, serves as evidence of the decades of research and development required to transform it into a viable drug [36]. The successful use of AMTs for oral delivery of GLP-1, exendin-4, oxyntomodulin, and aldafermin in various metabolic diseases (as shown in Table 1) serves as compelling evidence that such approaches represent a promising avenue for catalyzing the development of gastrointestinally derived peptides for treating metabolic diseases.

Challenges and strategies in advancing AMTs for delivery of therapeutic peptides

Oral delivery of peptides poses inherent challenges, including issues related to absorption and stability. As a result, we believe that the application of AMTs to improve the delivery of therapeutic peptides will become a significant focus within synthetic biology. However, developing AMTs poses a set of unique challenges, as the engineered strains must operate within the host's biological context [20,56,57]. Moreover, although the genetic toolbox for model organisms such as *E. coli* is extensive, the tools for engineering commensal and probiotic organisms, such as *Lactobacillus* and *Lactococcus*, are considerably more limited. As a result, AMTs stand as an application area of synthetic biology that demands innovative solutions. This will require integrating approaches aimed at improving the bioavailability of oral peptides, along with developing new solutions involving more robust engineered microbes, including programmable genetic circuits that can be controlled by environmental cues (Figure 2, Key figure). The subject of designing and constructing AMTs is extensive and rapidly evolving. For a more in-

Key figure

Schematic conceptualization of an advanced microbiome therapeutic (AMT)

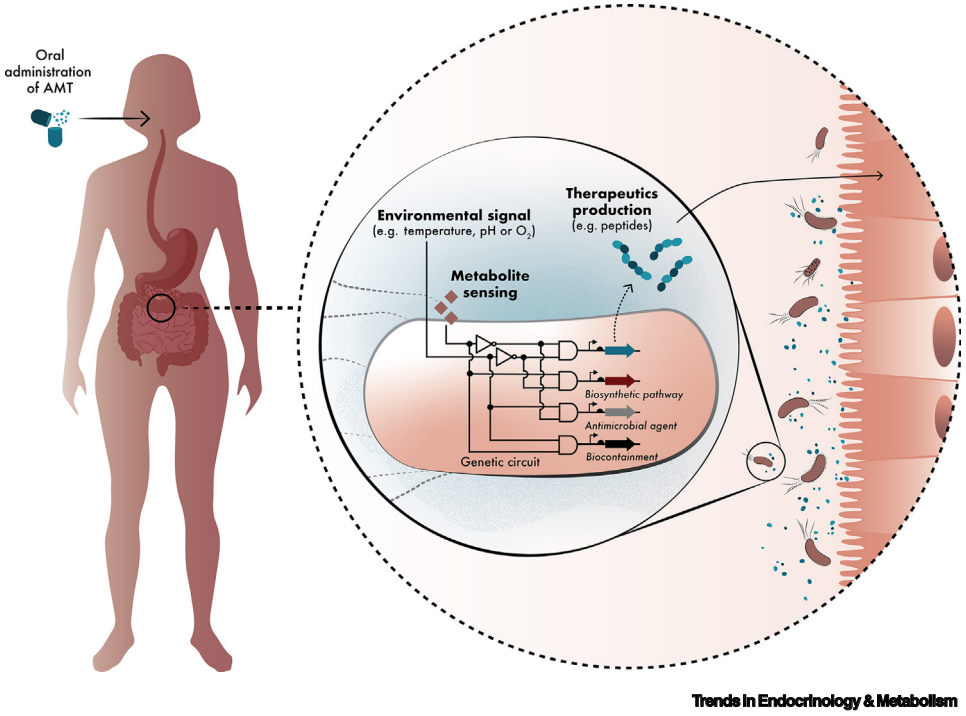

Figure 2. AMTs can be orally administered to deliver a therapeutic function directly within the gastrointestinal tract. In that manner, AMTs may encode multiple therapeutic functions, as well as fine-tune the dosing of therapeutics. This can be achieved by implementing genetic biosensors that regulate the expression of therapeutic agents in response to environmental signals, such as temperature, pH, and O_2, and other metabolites commonly encountered in the gastrointestinal environment [56]. AMTs can sense and respond to specific stimuli by expressing a therapeutic activity. General therapeutic strategies include the production of peptides and proteins, as well as encoding biosynthetic pathways to produce small molecules or antimicrobial agents. Additionally, an AMT might encode a function for biocontainment to reduce environmental and safety risks.

depth discussion on this challenge, readers are directed to other reviews [34,58]. In this section, we discuss the general challenges, as well as potential strategies to address them.

Enhancing peptide bioavailability

AMTs have shown promising results as a potential drug delivery platform in different metabolic diseases [22,44,55]. Yet, their application still poses similar challenges to other oral peptide hormone formulations concerning absorption and systemic exposure [19]. When peptide hormones are orally administered, they must be absorbed through the intestinal epithelial lining to enter the host's circulatory system. Although the body possesses various absorption pathways, the types of molecules that can pass through the epithelial barrier are limited, leading to low bioavailability [59–61].

To address these challenges in oral delivery of peptides, extensive research has focused on enhancing their bioavailability. Key strategies to enhance their oral bioavailability include (i) structural modification of the peptide and (ii) pharmaceutical formulations with permeability enhancers [62] and coupling drugs to molecules known for their facile absorption [29]. For instance, a formulation was successfully developed by researchers, incorporating the absorption enhancer sodium N-[8-(2-hydroxybenzoyl) aminocaprylate] (SNAC), to improve the bioavailability of semaglutide (a GLP-1 receptor agonist) [63]. Enhancing permeability using cell-penetrating peptides [28] has also been shown to effectively improve paracellular absorption by targeting tight junctions. AMTs may be engineered to produce such permeation enhancers, improving the bioavailability of the therapeutic peptides.

Furthermore, even though AMTs serve as a protective barrier against the harsh conditions in the intestine, the secreted protein must still endure the journey from the AMT to the epithelial cells. Consequently, there is a growing demand for more stable protein engineering to enhance the probability of protein absorption. Additionally, developing a strategy to direct AMTs to bind closely to epithelial cells could be beneficial, facilitating a more targeted delivery to the site of absorption in the host.

Robust colonization control

Most AMTs developed today employ noncolonizing probiotic microbes [64–66], which require regular dosing due to their transient presence in the gut. Probiotic microbes have mainly been used as AMTs because of their good history of safe use in humans and their manufacturing feasibility. Consequently, AMTs based on probiotic strains are meant to be eliminated from the body after the treatment course is completed, providing a safety benefit. However, investigating native commensal gut bacteria [67] offers exciting research possibilities. The isolation of host-specific microbial strains could lead to more robust colonization over time, because these isolated microbes have coevolved within the host's microbiota [68]. For instance, researchers have isolated a native *E. coli* chassis specific to the microbiota of conventionally raised mice. The strain was modified outside the host to overexpress a bile salt hydrolase. When reintroduced, it showed robust colonization for over 110 days and was effective in altering the bile acid profile of the host [68]. Furthermore, *in situ* genetic modification of specific microbes or broader communities directly in the gut could bypass the need to isolate specific strains and insert gene functions into one or more host microbes [69]. These approaches have the potential to not only enhance the robustness of the treatment but also improve patient compliance by reducing the frequency of dosing.

An understanding of ecological niches as well as engraftment within the intestine is crucial for advancing AMT-based therapies. The interindividual variation of the human microbiome, along

with factors such as ethnicity and diet, may lead to considerable variability in the colonization capacity of strains. Recent research has illuminated patterns of microbial colonization, highlighting the significance of metabolic factors such as carbohydrate and cofactor processing [70–72]. Employing a combination of methods, including the use of targeted antimicrobials or prebiotics, could improve the engraftment of therapeutic microbes into the microbiome, leading to more effective treatments. For instance, researchers have engineered an AMT to have unique access to the metabolic niche [73]. Supplying a specific nutrient facilitated the consistent and predictable engraftment of the introduced strain in mice. This approach even allowed the replacement of similar strains, demonstrating the potential of targeted strategies to enhance AMT colonization.

Stability and variability of the therapeutic activity

Overcoming genetic heterogeneity with engineered microbes in industrial fermentations remains a substantial challenge [74]. This issue is crucial not only for cell factories but also for AMT products during biomass production. Estimations for AMT suggest between ~50 and 100 generations may occur within the microbiota of the patient. Consequently, a total of 110–160 generations would occur from the original strain batch to the moment the AMT exits the body of the patient [75], ensuring that genetic stability becomes paramount during this period for the strain. Strategies such as synthetic addiction have proved effective in minimizing the emergence of non-producing mutants during scaled-up fermentation in cell factories [75,76]. However, AMTs also present significant challenges in maintaining the durability of engineered functions over time and across varying environments. Unfavorable environmental conditions and rapid changes can induce cellular stress and amplify evolutionary pressure [77]. This necessitates the development of more robust genetic circuits to ensure stability and effective therapeutic activity, as AMTs must maintain their function for extended periods of time.

Furthermore, control of the dose and activity from therapeutic interventions, especially those involving AMTs, is critical to ensuring that the peptide reaches the desired concentration in the target area. This involves controlling the rate of peptide production by the AMT [20,56,57] as well as the dynamics of AMT growth and clearance from the body. Although most optimization is conducted under ideal *in vitro* growth conditions, these may not accurately replicate the intended *in vivo* environment. As research progresses toward animal models and clinical applications, there is a need for more advanced *in vitro* systems, which better mimic the fluctuating conditions of the gut.

Safety and biocontainment

The risks associated with the release of live genetically modified organisms into the environment and their potential to proliferate beyond the initially targeted individuals cannot be disregarded. Although laboratory-developed genetically modified organisms typically exhibit lower fitness than their wild-type counterparts, the implications of unintended releases are substantial. Additionally, a chassis strain, which is the cellular platform used as a recipient of engineered biological systems in synthetic biology, may harbor antibiotic resistance genes and/or other genes with adverse effects on the host. For instance, colibactin in *E. coli* Nissle could potentially induce adverse effects on oncogenic mutations in humans [78]. Hence, the elimination of pathogenicity islands or detrimental functions must precede the transition of the chassis strain into clinical applications. These events require meticulous consideration in the development and deployment of AMT-based therapies, acknowledging the potential ecological and health consequences associated with the uncontrolled dissemination of genetically modified entities.

To guarantee the safe administration of AMTs, various biocontainment strategies have been investigated to inhibit the proliferation and survival of engineered microorganisms in unintended

environments. According to guidelines from the National Institutes of Health, an escape rate of fewer than 1 in 10^8 cells is considered to be acceptably safe [79]. Therefore, several precautions have been taken to develop biocontainment strategies, including the development of auxotrophic microbes [21,65,80], as well as environmentally sensitive strains [21] designed to prevent replication outside the gut. Readers are referred to other comprehensive reviews on design and safety considerations for AMTs for additional details on this subject [35,58].

Equally important is addressing the risk of unintended colonization, which demands the deployment of comprehensive strategies to prevent such occurrences. Conditional kill switches have been engineered to either eliminate the engineered microbes or destroy their genetic circuits [81,82]. These approaches have proved to be effective, particularly in preclinical testing. In early clinical trials, auxotrophy has been the sole biocontainment method implemented for AMTs [83,84]. Looking ahead, researchers and regulatory bodies need to work together in advancing the necessary technology for the safe deployment of AMTs.

Concluding remarks and future perspectives

While AMTs hold promise with potential advantages over traditional drug administration methods, such as improved bioavailability of therapeutic peptides, most developments are still in the preclinical stage. Therefore, it is essential to evaluate their efficacy in clinical trials compared with traditional drugs. Nevertheless, in this last section, we highlight several emerging opportunities for AMTs to address various challenges associated with the oral delivery of peptides. For instance, AMTs might offer a more dynamic treatment regime than injectables and conventional oral strategies. Because of the multiple factors involved in metabolic diseases, it is likely that, in the future, patients will require a combination of multiple therapeutics that can adapt to their particular condition. AMTs may be fine-tuned to dose the necessary levels on the basis of robust promoters for expression *in vivo* [20,57] and could be engineered to sense and respond to specific environmental and disease signals, allowing adaptive combinatorial therapeutic regimens. A variety of biosensors tailored for AMTs have been developed to control gene expression within the gastrointestinal tract [34,56,85,86]. Upon the integration of these advanced strategies, the engineered strain gains the ability to produce the therapeutic payload exclusively when needed, allowing dynamic precise administration of the peptides.

On the basis of the success of GLP-1 in managing metabolic diseases, several peptides are quickly moving into clinical trials to bring the next generation of therapeutic peptides. Peptides such as oxyntomodulin [87], amylin [88], PYY [89], GIP [90], and glucagon [90] contribute to satiety and metabolism regulation (Figure 1). Although GLP-1 receptor agonists such as liraglutide and semaglutide have been successfully formulated into pharmaceuticals, many other peptides remain unexplored in this context. In that sense, AMTs could be used as a tool to accelerate the evaluation of novel peptides and their effect on metabolic diseases.

Additionally, considering combination therapies, an AMT may deliver more than one peptide or fusion tandem peptides with dual functions. The emergence of tandem peptides, exemplified by tirzepatide, a GIP/GLP-1 tandem peptide [91], further broadens the horizons of AMT applications for managing metabolic diseases.

By employing novel approaches such as CRISPR-Cas [92], researchers can efficiently generate a multitude of strain variants through automated computationally guided design, cloning, and screening. The accelerated generation of AMT strains will rapidly surpass the capacity of testing strategies, such as those reliant on animals. Therefore, testing for efficacy and safety is likely to become the bottleneck in the clinical translation of these therapies. To address this, the establishment

Outstanding questions

How can we successfully translate AMTs to clinical applications?

What other strategies can be implemented in combination with AMTs to improve the stability and absorption of peptides across intestinal barriers?

Considering interindividual variation in the gut microbiome, what strategies can be devised to ensure robust colonization control of AMTs?

How consistently can AMTs maintain therapeutic activity over long periods of time within the dynamic environment of the gut microbiota?

What measures should be implemented to ensure the safety of AMT applications?

What are the limitations and opportunities for applying AMTs in diverse medical areas beyond metabolic diseases?

of robust *in vitro* models is crucial for expediting the design, build, and test cycle. Novel *in vitro* models, such as organ-on-a-chip [93] or more advanced ones, such as HuMiX [94], can play pivotal roles in studying microbe–host interactions and accelerating the preclinical development phase through *in vitro* assays. These tests can help screen and assess candidate strains before moving to animal testing. However, animal models are still required to ensure a thorough evaluation of safety before moving to clinical trials.

The adoption of AMTs may potentially alleviate socioeconomic challenges as peptide-based medicines, particularly complex ones, can be expensive to synthesize. This cost impacts downstream processing of the recombinant protein and manufacturing, ultimately affecting the final affordability for patients. However, further technoeconomic analysis of AMTs compared with traditional peptide manufacturing is still required. This analysis is highly relevant to consider when addressing health issues that disproportionately affect racial and ethnic minority populations and those of lower socioeconomic status, including obesity and its downstream consequences such as type 2 diabetes [95,96]. AMTs offer a potential solution by making the manufacturing of therapeutic peptides more affordable, as microbial biomass production is comparatively easier and requires less downstream processing than more traditional peptide manufacturing.

Finally, the scope of this review primarily focused on the application of AMTs for delivering of gut-derived hormones and peptides. However, it is important to note that AMTs also hold promise in delivering small molecules to treat metabolic diseases [97,98]. In addition, researchers have explored the potential applications of AMTs in other areas, such as for treating intestinal inflammation [65,84,99], wound healing [100], deficient nutrient metabolism [66], and even direct injection into the tumor microenvironment for cancer treatment [101–104]. Although challenges in AMT development remain (see Outstanding questions), multiple AMTs have reached clinical development for indications ranging from Crohn's disease [84], type 1 diabetes [105], cancer [106], and phenylketonuria [107]. With the rapid development of synthetic biology approaches, the future of AMTs holds immense promise, with a multitude of potential applications waiting to be uncovered and harnessed for the benefit of medical science and enhanced patient care.

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Declaration of interests

M.N. is founder and board member on the Scientific Advisory Board of Caelus Pharmaceuticals and Advanced Microbiome Interventions, The Netherlands. However, none of these bear direct relevance to the present article. M.O.A.S. is a cofounder and board member of Clinical-Microbiomics and SNIPR biome and a board member of Novonesis. However, none of these bear direct relevance to the present article. The remaining authors have no interests to declare.

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