Advances in oral peptide therapeutics

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Abstract | Protein and peptide therapeutics require parenteral administration, which can be a deterrent to medication adherence. For this reason, there have been extensive efforts to develop alternative delivery strategies, particularly for peptides such as insulin that are used to treat endocrine disorders. Oral delivery is especially desirable, but it faces substantial barriers related to the structural organization and physiological function of the gastrointestinal tract. This article highlights strategies designed to overcome these barriers, including permeation enhancers, inhibitors of gut enzymes, and mucus-penetrating and cell-penetrating peptides. It then focuses on the experience with oral peptides that have reached clinical trials, including insulin, calcitonin, parathyroid hormone and vasopressin, with an emphasis on the advances that have recently led to the landmark approval of an oral formulation of the glucagon-like peptide 1 receptor agonist semaglutide for the treatment of type 2 diabetes.

The medicinal utility of biologic therapeutics — encompassing large proteins, antibodies, hybrid fusion proteins, antibody–drug conjugates and therapeutic peptides — continues to expand. Such therapies are typically administered by parenteral injection because of their poor oral bioavailability. Although this is less of a barrier in the context of acute administration for limited time periods, daily injections of therapeutics such as insulin for decades may pose a challenge for medication adherence. Commonly voiced issues include aversion to injections, concerns about needle size or discomfort at the injection site[1], and persistence with injections remains suboptimal, even with once-weekly therapies[2].

Consequently, extensive efforts have explored the feasibility of delivering proteins via alternative routes, including formulations suitable for oral, nasal, ophthalmic, pulmonary, buccal and transdermal administration[3]. The widespread use and convenience of oral drug delivery makes this route particularly attractive for patients. However, the substantial challenges of oral biologic delivery become evident when considering the anatomical structure and function of the gastrointestinal tract (FIG. 1). These include the enzymatic breakdown of ingested complex nutrients such as proteins into easily absorbed smaller molecules, as well as restricting the access of toxins and microbial pathogens, which is achieved by cellular and mucosal barriers.

This article focuses on developments in oral peptide therapeutics — arbitrarily defined as smaller proteins with a molecular mass of <9,000 Da[4] — as these provide more feasible opportunities for oral delivery than do larger proteins[5]. Although there have been efforts to develop approaches to optimizing the pharmaceutical characteristics of peptides — including in silico models that incorporate size and molecular features such as hydrogen-bonding capacity, lipophilicity, cyclization and polar surface area[5,6] — currently no validated framework can be generally applied to the rational development of an oral formulation of a given peptide. Furthermore, the oral delivery technologies for which success has been reported in preclinical models have often failed to translate into sufficient oral bioavailability in clinical trials. The aim of this article is not to comprehensively discuss such technologies, and readers are referred to other recent reviews for further analysis of oral delivery technologies[3,4,7]. Rather, after briefly discussing the barriers to oral peptide delivery and selected approaches to addressing them, this article focuses on the experiences with oral formulations of peptides such as insulin that have been investigated in clinical trials. In particular, the recent FDA approval of an oral formulation of the glucagon-like peptide 1 (GLP1) receptor agonist semaglutide for the treatment of type 2 diabetes (T2D) is a landmark in the field that could energize the development of oral peptide therapeutics.

Barriers to oral peptide delivery

Peptide transit across the intestinal epithelium, followed by secretion into the lymphatic system or bloodstream of a substantially non-degraded therapeutic, requires the circumvention of multiple structural and functional barriers, which are summarized briefly here and shown in FIG. 1. Readers are referred to other, comprehensive reviews for more extensive discussion of these barriers[4,8,9].

Protein-degrading enzymes. Ingested peptide formulations first encounter digestive enzymes, including amylase and lipase, in the saliva. Upon entry into the stomach, the ingested peptides encounter a low pH and enzymes such as pepsin and cathepsin that are highly efficient at proteolysis. Additional proteolytic enzymes are present in the lumen of the small intestine, including those emanating from pancreatic secretions, as well as brush border membrane peptidases expressed by enterocytes. These include trypsin, chymotrypsin, carboxypeptidase and numerous dipeptidases and aminopeptidases[10].

The cell or mucosal models commonly used to examine the efficiency of drug transport across epithelial cells[11] may not fully mimic the complex enzymatic environment, including differences in regional pH, within the gut lumen, and thus lead to overestimation of peptide bioavailability. Wang and colleagues assessed the stability and enzymatic degradation of 17 different peptide-based drugs using gastric and small-intestinal fluids from pigs and humans, as well as enzyme-supplemented (pepsin and pancreatin) simulated fluids. Several of the peptides examined (somatostatin, calcitonin, glucagon, secretin and insulin) were at least 12 amino acids or longer[12]. Small peptides, including those with cyclic structures, were generally more stable,

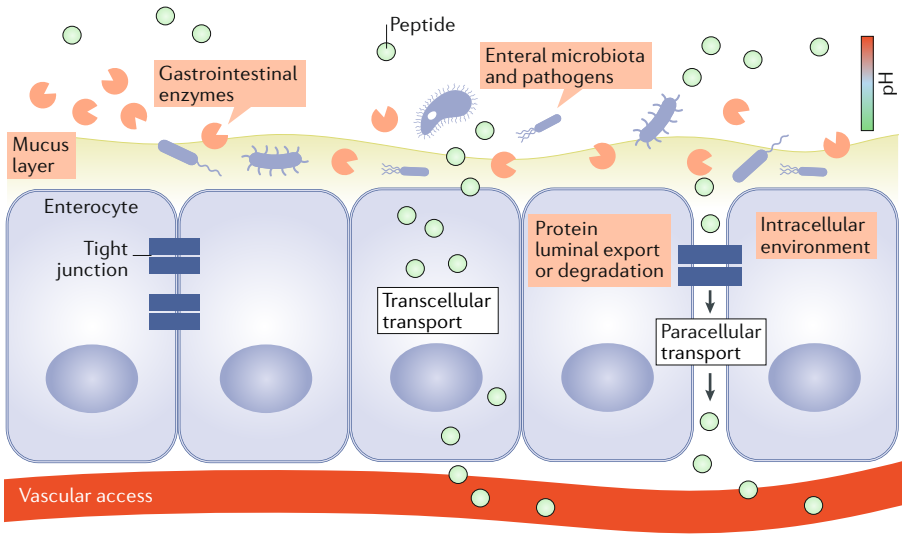

Fig. 1 | **Schematic representation of intestinal structure and the associated challenges to efficient oral absorption of peptides.** The challenges of oral peptide delivery become evident when considering the normal physiological roles of the gastrointestinal tract. Ingested proteins are efficiently broken down, via a range of pH-sensitive proteases, into amino acids, which are then actively transported across the mucosa via a complex family of transporter proteins. Similar digestive processes ensure the enzymatic breakdown of complex carbohydrates and lipids into smaller, easily absorbed molecules. A secondary and simultaneous function of the gut epithelium is the recognition and exclusion of toxins, bacteria, viruses and related microbial pathogens. Hence, the lining of the gastrointestinal tract has evolved a sophisticated set of cellular and mucus barriers to restrict the access of foreign and noxious agents. Various strategies have been pursued to overcome these barriers (highlighted in orange) to enable peptide drug transit across the intestinal epithelium and secretion into the lymphatic system or bloodstream, which are summarized in FIG. 2. Paracellular approaches are designed to enable the passage of a peptide drug through transient disruption of cellular junctions, whereas transcellular approaches facilitate the passage of peptide drugs through the cytoplasm of enterocytes, followed by secretion into the lymphatic or systemic circulation. Image adapted with permission from REF.[54], Elsevier.

whereas larger peptides were more rapidly degraded. Although this is less extensively characterized, the local intestinal microbiota are known to influence drug metabolism, and may also liberate enzymes contributing to peptide degradation[13].

The gastrointestinal mucus layer. A complex mucus layer, composed of cell-associated mucins and glycoproteins, lies adjacent to absorptive enterocytes within the epithelial mucosa of the stomach and bowel, serving as both a lubricant for ingested nutrients and a physicochemical barrier that traps larger molecules and pathogens[14]. Within the stomach, the pH of the mucus layer ranges from below 2 at the luminal surface, to a neutral pH approximating the epithelial surface[15], and mucin turnover is highly dynamic, reflecting degradation in the acidic and enzyme-rich environment, balanced by constant resynthesis of new mucus[15]. The ~20 different mucin proteins may be either secreted or cell-associated molecules with short cytoplasmic tails that enable intracellular signal transduction[15]. Secreted mucins are linked together via disulfide bonds to form macromolecules that are highly glycosylated, which enables stabilization of the mucin complex and protection against enzymatic degradation. The size and structure of mucus, together with the presence of intermolecular covalent and non-covalent interactions, dictate the diffusion of peptides and larger molecules through the mucus layer. Mucus also serves as a reservoir for antibacterial defensins and physically traps microorganisms in outer superficial mucus layers[16], preventing the direct access of most bacterial species, as well as large molecules, to the enterocyte surface. Some mucins, exemplified by MUC2, also control the extent of luminal bacterial colonization through modulation of dendritic cell activity, leading to the suppression of inflammatory responses and the augmentation of tolerogenic responses in the gut mucosa[17].

Extensive studies of biosimilar and naturally occurring mucus in both cell systems and cell-free models have established the principles determining hydrophobic drug–mucus interactions and the importance of peptide size and charge, as well as mucus pore size, which in turn influence mobility and permeation through experimental mucus systems[18]. However, the extent to which studies of peptide drug permeation through experimental mucus systems modelled ex vivo have recapitulated the complexity of the mucous layer in vivo remains uncertain.

Paracellular or transcellular routes. The passage of orally ingested peptide drugs across the gut epithelium involves either navigation of the intercellular spaces surrounding and between cells (the paracellular route) or drug transit through cells (the transcellular route), so as to exit intact into the vascular space adjacent to the basolateral surface of the gut epithelium (FIG. 1).

Transit through the paracellular route is challenging because of a complex series of molecular barriers, including tight junctions (zonula occludens), adherens junctions (zonula adherens) and desmosomes (macula adherens)[19]. Collectively, these junctions encircle epithelial cells and maintain epithelial structure and integrity. Junctions permit the necessary flux of water, ions and solutes, while selectively excluding toxins, macromolecules and microorganisms[20]. Occludens, claudins and junctional adhesion molecules form highly regulated and dynamic complexes, directly linked to the actin–myosin cytoskeleton[21]. While transient disruption of junctional complexes facilitates peptide absorption from the gut lumen, sustained functional impairment of tight junction complexes by drugs, toxins or infections has been linked to systemic endotoxaemia and gastrointestinal inflammation[22].

The passage of drugs through the transcellular pathway presents a different set of challenges. Absorptive enterocytes have a specialized system of molecular carriers to transport amino acids, sugars, fatty acids, bile acids and related essential micronutrients from the lumen to the basolateral surface and circulation[23,24]. However, a series of intracellular pathways simultaneously promote efficient targeting of foreign intracellular proteins to intracellular lysosomal pathways[25], leading to degradation. Alternatively, foreign intracellular proteins may be rerouted back to the mucosal surface for luminal, rather than basolateral, secretion. Additional challenges for oral peptide absorption include a lack of receptors for peptides on the luminal surface of enterocytes and the absence of pathways facilitating the uptake of luminal proteins.

Inter-individual variability. Another major consideration for development of an oral peptide is the tremendous inter-individual variability in the physiology of the gastrointestinal tract[26], including the extent of mucus and enzyme production and control of gastric emptying and gut motility. The rate of gastrointestinal transit is a key factor determining epithelial exposure to an ingested peptide and may be influenced by the size, composition and timing of meal ingestion, as well as by ageing. Variability in gut motility and differential rates of absorption are particularly relevant to the development of therapeutics such as insulin for diabetes; individuals with mild dysglycaemia or diabetes may have dysregulation of gut motility[27], and even suboptimal glycaemic control leads to impaired gastric emptying in individuals with type 1 diabetes (T1D) or T2D[28]. Moreover, inter-individual differences in the luminal epithelial environment, including the relative expression of digestive enzymes, peptidases and relevant transporters, potentially contribute to the timing and extent of transmucosal drug absorption[29].

Approaches to oral peptide delivery

Key considerations in evaluating the suitability of oral peptide formulations include the extent of absorption across the intestinal mucosa, the achievement of detectable pharmacodynamic activity and systemic bioavailability. If bioavailability is too low, the cost of the manufactured drug product may be excessive, and commercial development may not be viable.

Multiple strategies to facilitate oral peptide delivery are being pursued, often in combination, including permeation enhancers, methods to combat enzymatic degradation, nanoparticle carriers, intestinal patches and microneedle delivery devices (FIG. 2). These approaches have been reviewed recently[3], so this section focuses on representative technologies, with an emphasis on those that have reached clinical trials or have strong promise to move into clinical evaluation.

Permeation enhancers. Permeation enhancers[4] can target either the transcellular route, by facilitating the passage of non-degraded peptides through epithelial cells, or the paracellular route, via interference with the function of intercellular junctional and adhesion proteins — for example, with chelators that sequester calcium[30], which is simultaneously needed for E-cadherin function and as a cofactor for some proteases.

Enhancers must achieve sufficient yet transient disruption of the intestinal epithelium to enable meaningful peptide absorption, while maintaining an acceptable safety profile and minimal local or systemic toxicity. Screening for agents that enhance permeability generally employs isolated gut mucosal segments studied ex vivo, or gut epithelial cell lines, analysed using Ussing chambers. This approach enables the measurement of drug transport across cell layers, as well as indirect assessment of cell and tight junction integrity through an analysis of transepithelial electrical resistance[31]. For example, Whitehead and colleagues analysed the properties of several dozen permeability enhancers in assays using intestinal epithelial Caco-2 cells and found that the chemical structure of specific compounds correlated in a general manner with their mechanisms of action; fatty esters predominantly enhanced paracellular permeability, whereas zwitterionic and cationic surfactants augmented transcellular permeability[32]. Permeation enhancers with different molecular mechanisms of action may be combined and used together at lower doses, to minimize cell toxicity while achieving synergistic enhancement of permeation efficiency[33]. The development of reconstituted human intestinal tissue or gut organoids with functional tight junctions[34] holds promise for the rapid screening of permeability enhancers that safely and transiently modify epithelial permeability.

While numerous permeation enhancers have exhibited efficacy in preclinical studies, only a few enhancers have shown sufficient safety and efficacy to progress into clinical trials[35]. Of these, two permeation enhancers — sodium caprate (C_{10}; also known as decanoic acid) and sodium *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC; also known as salcaprozate sodium) — have been used in proprietary delivery platforms for many years and have been tested more extensively in humans than any other enhancers[36]. Sodium caprate, a medium-chain fatty acid that is approved as a food additive, exists in an ionized soluble form with detergent capacity at pH values that typically occur in the small intestine. Its mode of action is thought to have both transcellular aspects, based on epithelial plasma membrane interactions, and paracellular aspects, based on effects on junctional proteins[36]. SNAC, which was identified on the basis of observations that acylated amino acids formed microspheres that facilitated the oral absorption of small peptides[37], has 'generally recognized as safe' (GRAS) status. SNAC was also originally thought to enhance transcellular and paracellular permeability, but recent studies have highlighted a predominant transcellular mode of transit through the gastric epithelium for semaglutide co-formulated with SNAC[38], which is discussed in greater depth below. 8-(*N*-2-hydroxy-5-chloro-benzoyl)-amino-caprylate (5-CNAC) is another acylated amino acid that has been tested in clinical trials — for example, with calcitonin (see below). It is rapidly absorbed and metabolized in humans, with renal excretion representing the predominant mode of metabolite clearance[39].

Ideally, permeation enhancers comprise simple, easy-to-manufacture and safe ingredients that can be utilized, using a range of formulations, to enhance the absorption of multiple proteins, without substantial need for alteration of the core components of clinically validated enhancers[36]. Multiple theoretical concerns include the potential for enhanced

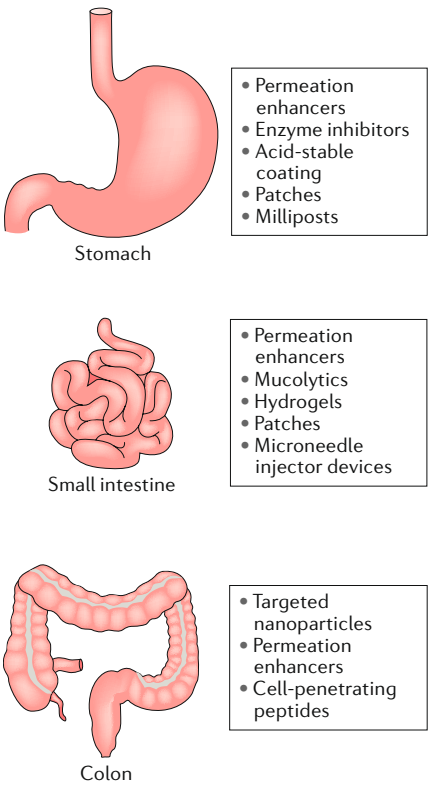

Fig. 2 | **Technologies that have been applied for gastrointestinal peptide absorption in different regions of the gastrointestinal tract.** Examples of potential approaches enabling regional targeting of orally administered peptide drugs are shown in the boxes next to the stomach, small intestine and bowel. Among these strategies, permeation enhancers have been the most extensively developed, and a permeation enhancer is a component of the recently approved oral formulation of semaglutide, summarized in FIG. 3.

absorption of noxious agents (bacteria, fungi, viruses and toxins), development of immune responses or alteration of the gut microbiome through the use of permeability enhancers. However, such toxicities have not yet emerged as problems in routine monitoring of adverse events in clinical trials[4,40]. For example, in the largest trial to date examining the safety of an oral insulin, while more subjects treated with an insulin analogue encapsulated with sodium caprate, known as I338, developed human anti-insulin and cross-reacting antibodies (18 versus 5 in the placebo group), the small numbers of patients with antibodies and a lack of associated adverse events precluded meaningful conclusions about the clinical significance of these antibodies[41]. Similarly, no imbalance of serious adverse events was detected in the largest trial of oral semaglutide, PIONEER 6, which was designed to examine the long-term cardiovascular safety of oral semaglutide co-formulated with SNAC (see below)[42]. The success and relative safety of several permeation enhancers in the clinic has fostered great interest in exploring opportunities for further enhancing the efficiency of transmucosal passage of protein therapeutics.

Modulation of pH. Pepsin in the acidic environment of the stomach readily cleaves multiple peptides. Tablets containing peptides for oral delivery can be coated with an acid-stable enteric coat to prevent their dissolution in the stomach. Once a tablet leaves the stomach and reaches the upper intestine, the elevation in pH results in dissolution of the enteric coat and release of the tablet contents, as is illustrated for an oral formulation of calcitonin that has been tested in clinical trials[43] (see below). Intestinal and pancreatic enzymes are also capable of rapidly degrading peptides. The optimal pH for these gastrointestinal enzymes is neutral to basic; the inclusion of citric acid in the tablet results in a local, transient decrease in pH, resulting in inhibition of the resident peptidases. Indeed, co-administration of citric acid together with oral salmon calcitonin is presumed to enhance bioavailability in part by reducing the activity of local tryptic enzymes, resulting in enhanced absorption of oral salmon calcitonin in beagles[44].

Direct enzyme inhibition. Another strategy to circumvent gut enzyme activity is direct enzyme inhibition. Enzyme inhibitors including aprotinin, soybean trypsin inhibitor and leupeptin have been co-formulated together with peptide cargo to provide localized protection of the peptide at the site of mucosal absorption[45]. The most clinically advanced example of direct enzyme inhibition as a component of an oral peptide formulation may be an oral insulin formulation developed by Oramed, known as ORMD-0801 (see below)[46]. This formulation includes soybean trypsin inhibitor as well as a chelating agent that scavenges calcium, which is a cofactor for many proteases. However, the utility and safety of enzyme inhibition as a general strategy for enhancing oral peptide absorption is uncertain.

Peptide cyclization. Cyclization strategies remove exposed N and C termini from peptides, which are particularly susceptible to enzymatic cleavage. A cyclic structure is a feature of many naturally occurring small peptides, including two that have been successfully developed for oral administration: the immunomodulatory drug cyclosporine[47], for which an oral microemulsion branded Neoral was approved in the 1990s, and desmopressin, an analogue of the peptide hormone vasopressin that has greater resistance to enzymatic degradation than does vasopressin[48] (see below). The extent to which cyclization is a meaningful and generalizable strategy to enhance oral peptide absorption is not yet clear. Nielsen and colleagues recently reported the physicochemical parameters and oral absorption properties of 125 cyclic peptides, noting that the majority of these small peptides had limited oral bioavailability, and most had been examined in only a small number of preclinical and even fewer clinical studies[49].

Mucus-penetrating agents. Several strategies have co-formulated peptides with mucolytic agents or mucus-penetrating agents to enhance the rate of passage of peptides across the mucus barrier. In preclinical studies, the addition of hydrophilic polymers such as polyethylene glycol (PEG) chains enhances mucus penetration, as does co-formulation of peptides such as insulin within nanoparticles containing a hydrophilic coating of 2-hydroxypropyl methacrylamide copolymer (pHPMA) derivatives[50]. Self-nanoemulsifying drug delivery systems (SNEDDS) have also been tested that contain mixtures of oil, surfactant and cosurfactant[51]. Insulin delivery in preclinical studies in rats has also been achieved through the development of mucoadhesive *N*-trimethyl chitosan chloride-coated PLGA nanoparticles containing cell-penetrating peptide (CPP)–insulin conjugates[52]. While mucus-penetrating strategies continue to be studied extensively, their safety and efficacy have not yet been validated in large clinical trials[53].

Cell-penetrating peptides. CPPs may be peptide sequences derived from viruses that are normally efficient at membrane translocation or cell entry, non-viral proteins or smaller molecules such as octa-arginine[54,55]. CPPs may interact with membrane glycosaminoglycans, traversing endocytic pathways and ultimately delivering their protein cargo to the systemic circulation via exocytosis. Alternatively, CPPs may facilitate cell entry by traversing membrane lipid bilayers via energy-independent mechanisms[54]. A large number of energy-dependent and energy-independent pathways and mechanisms have been identified as candidates facilitating the entry of CPPs and their cargo, and the efficiency of the pathways engaged may be highly CPP, cargo and cell-type specific[54]. CPPs have been explored for the delivery of anticancer and antimicrobial therapies and as imaging agents, but their use for enhancing the oral absorption of peptide therapies has not yet been validated in the clinic.

Intestinal patches. Intestinal patches for oral drug delivery physically protect a small reservoir of drug from local degradation while positioning the drug close to the absorptive epithelium. For example, a mucoadhesive patch was created by compressing a polymeric matrix containing carbopol, pectin, sodium carboxymethylcellulose and salmon calcitonin and coating this matrix with ethyl cellulose on all but one side. The patch adhered to the small intestine mucosa in pigs and rats and released salmon calcitonin that was detectable in the systemic circulation and was associated with a reduction in blood calcium in rats[56]. More recently, a mucoadhesive patch that exploits iontophoresis to disrupt intestinal tight junctions and facilitate paracellular drug transport has been reported, which delivered insulin into the systemic circulation of rats following a brief period of electric current, without evidence of major structural impairment of the local gut mucosa[57]. While progress continues in patch technology for oral drug delivery, clinical proof of concept has not yet been forthcoming.

Hydrogels. Multiple hydrogel formulations have been explored for enhancing intestinal absorption of peptides from both the small and the large bowels (for example, REFS[58–60]). Hydrogels contain water, a crosslinked polymer and a protein cargo, and potentially also mucoadhesive polymers, to facilitate prolonged retention and enable a prolonged peptide residency time within specific gut regions, while simultaneously resisting enzymatic degradation. So far, however, hydrogels for therapeutic peptide delivery have not made meaningful progress towards the clinic.

Microneedle devices and milliposts. The inherent attractiveness of device-based delivery technologies reflects their generalized suitability for delivering a broad range of peptides and proteins, with fewer limitations based on protein size. Rani Therapeutics is developing a small enteric-coated capsule for the delivery of biological therapeutics, including peptides. The capsule undergoes pH-dependent dissolution in the small intestine, with generation of carbon dioxide inflating a small balloon that in turn positions a microneedle-based device adjacent to the epithelium for mucosal injection of therapeutic cargo[61]. Preclinical studies have demonstrated that the capsule can deliver up to 3,000 μg of drug, equivalent to ~80 units of insulin, within 30 min of capsule deployment within the gut[61]. The company has reported that the safety of the capsule device (known as the RaniPill), without active drug substance, has been successfully tested in humans following administration in either the fasted or fed state, and clinical trials using octreotide have been planned (see the Rani Therapeutics press release in the Related links).

Preclinical studies with two innovative devices have been reported recently by Abramson and colleagues[62,63]. An orally dosed microneedle delivery device, designated a luminal unfolding microneedle injector (LUMI), contains multiple drug-loaded microneedles encapsulated within a poly(methacrylic acid-co-ethyl acrylate) and PEG coating, and is designed to dissolve at pH levels encountered in the small intestine. Following delivery of the device into the swine gut, the capsule dissolved, liberating spring-enabled biodegradable microneedles loaded with insulin, which penetrated the gut mucosa. Plasma insulin levels increased and glucose decreased within 15–30 min, with a systemic bioavailability of co-formulated insulin of more than 10% without histological evidence of intestinal perforation[62]. Abramson and colleagues also described an orally ingested self-orienting millimetre-scale applicator (SOMA) that adheres to gastric mucosa and delivers, via injection, pharmaceutical products such as insulin, without puncturing the outer layer of the stomach. A compressed mixture containing insulin and polyethylene oxide was delivered from the SOMA device via milliposts containing biodegradable polymers, resulting in detectable levels of insulin associated with progressive reduction of blood glucose in non-diabetic swine, without histological or functional evidence of gastrointestinal injury[63]. The extent to which LUMI or SOMA devices can be safely and reproducibly targeted to the appropriate regions of the gastrointestinal epithelium following oral ingestion requires further investigation.

Oral peptides assessed in humans

So far, endocrine disorders have been a strong focus of efforts to deliver oral peptide therapies. Analogues of vasopressin, calcitonin, insulin, somatostatin, parathyroid hormone (PTH), thyroid hormone-releasing hormone, uroguanylin and GLP1 have all been formulated for oral administration[35]. Progress in the development of oral peptide therapies that have been tested in humans is highlighted in this section.

Vasopressin. A nonapeptide (M_r = 1,069) with six amino acids in a ring structure, joined by a disulfide bridge and a three-amino-acid tail, vasopressin was among the first peptide hormones developed for oral administration. Originally described as a hormone with vasoconstrictor activity, vasopressin, also known as antidiuretic hormone, is made in the hypothalamus, is released from the posterior pituitary and functions as a potent regulator of water absorption in the kidney, thereby decreasing urine output[64]. Individuals with loss of central vasopressin secretion (central diabetes insipidus) or with nocturnal enuresis are candidates for vasopressin therapy.

Desmopressin, a modified analogue of vasopressin with deamination of the first amino acid and substitution of the eighth amino acid, L-arginine, with D-arginine, was developed in tablet form for the treatment of diabetes insipidus in the 1980s. Desmopressin has much greater antidiuretic activity than native vasopressin, and very little vasoconstrictive activity[65,66]. Desmopressin also has a prolonged antidiuretic action relative to native vasopressin, due in part to its resistance to degradation by vasopressinase[64,66]. In clinical trials, oral desmopressin rapidly reduced urine volumes in a dose-dependent manner. Several-fold higher doses of oral desmopressin relative to intranasal dosing, and ~200-fold higher relative to subcutaneous dosing, were required in order to achieve effective antidiuresis[67]. Oral desmopressin is approved for the treatment of central diabetes insipidus and primary nocturnal enuresis, whereas injectable desmopressin has been favoured for use in individuals with clotting disorders, due to its actions of increasing the circulating levels and activity of the clotting proteins factor VIII and von Willebrand factor[68]. However, in the context of oral peptides in general, desmopressin can be viewed as an exception, as its cyclic nature is atypical, and its exceptional potency means that a bioavailability of only 0.17% for the oral formulation, Minirin, is still viable[36].

Insulin. Insulin (M_r = 5,808), the most widely used injectable peptide therapeutic, is currently available as rapid-acting formulations, as well as longer-acting basal formulations suitable for once-daily administration. As insulin is used by millions of people with diabetes worldwide, it has received considerable attention as a candidate peptide for oral delivery. As well as reducing the burden of injections, oral delivery could theoretically mimic a more physiological route of insulin delivery to the liver via the portal system, although the long-term benefit or safety of liver-targeted insulin remains unknown[46]. However, the development of a rapid-acting oral insulin formulation is highly challenging, given inter-individual and intra-individual variability in rates of gastric emptying[28] and the timing of food ingestion, as well as the risk of differences in pharmacodynamic responses, potentially enhancing risks of hypoglycaemia[69]. Furthermore, some individuals with obesity and/or diabetes have impaired intestinal expression of tight junction proteins, defective intestinal barrier function and a 'leaky gut'[70,71], suggesting that inter-individual differences in gastrointestinal permeability may also influence the delivery of oral peptides. Oral delivery of a long-acting basal insulin may be more feasible.

Several hundred studies have described chemical and technical approaches to the oral delivery of insulin, targeting the stomach, small intestine or colon, using a wide range of drug delivery systems (reviewed in[7,72]). The majority of the studies have demonstrated glucose-lowering

effects of an externally administered insulin preparation in animals, often with only a single dose assessed. Comparatively few studies have examined the feasibility of chronic oral insulin dosing in diabetic animals for weeks to months, and only a handful of technologies have progressed beyond phase I human testing. Selected agents that have reached clinical trials are discussed below, as well as selected emerging preclinical technologies.

Insulin has been formulated with a number of permeation enhancers for oral delivery. Among the enhancers highlighted above, SNAC has not been pursued, because higher concentrations of SNAC attenuate the glucose-lowering response of insulin[73], but sodium caprate has been extensively studied in the clinic. For example, Halberg and colleagues compared a long-acting basal insulin analogue formulated in a tablet with sodium caprate developed by Novo Nordisk, known as I338, for once-daily administration against once-daily subcutaneous injections of insulin glargine over 8 weeks in a phase II trial involving 50 individuals with T2D[41]. Reductions in fasting glucose, the primary trial end point, were similar in both treatment arms, and no imbalance in rates of adverse events was detected between groups[41]. Despite these promising results, the clinical development of I338 was discontinued, largely due to low bioavailability, which was estimated at 1.5–2%. Intriguingly, higher insulin antibody titres were detected in subjects treated with I338 versus those receiving insulin glargine, without evidence of associated adverse events or reduced clinical efficacy[41].

Biocon has designed insulin tregopil, a human insulin analogue with a methoxy-triethylene-glycol-propionyl moiety linked to the Lys-β29 amino group and formulated with sodium caprate, as a fast-acting agent to reduce postprandial hyperglycaemia[74]. Dose-ranging studies have demonstrated dose-proportional increases in plasma insulin levels coupled with corresponding reductions of blood glucose following acute administration of 10–30-mg tablets[75]. Additional studies testing 30 or 45 mg of insulin tregopil versus insulin aspart in subjects with T2D have been completed (ClinicalTrials.gov registration number NCT03430856), but the study results have not yet been reported. The bioavailability of insulin tregopil was estimated to range from 0.82% to 0.85% in dog studies[76].

ORMD-0801 is a formulation of native insulin with a non-disclosed permeation enhancer, soybean trypsin inhibitor and a chelator[46] that has been assessed in subjects with either T1D[77] or T2D[78]. In an open-label study of eight individuals with T1D, ORMD-0801 three times daily for 10 days reduced the mean 24-h glucose area under the curve (AUC) by 17%, while rates of hypoglycaemia trended higher than pretreatment baseline data[77]. In a phase II dose-ranging study, 31 adult patients with T2D treated with ORMD-0801 showed improvements in mean placebo-subtracted AUC glucose ranging from −7.65 mg dl^{-1}, for once-daily dosing, to −9.91 mg dl^{-1}, for three-times-daily dosing[78]. No difference in rates of hypoglycaemia was noted when comparing placebo with ORMD-801-treated subjects, and no serious adverse events were reported[78]. ORMD-0801 continues to be evaluated in separate clinical trials of subjects with T1D and T2D. The bioavailability of ORMD-0801 was estimated at 5–8% from studies in beagle dogs[46].

Diasome has reported clinical testing of a liver-targeted insulin, formulated for both subcutaneous and oral administration[79]. The oral formulation consists of vesicles that carry insulin and a proprietary hepatocyte-targeting molecule in the phospholipid bilayer, which act to protect insulin from proteolytic degradation. The oral HDV-1 insulin formulation reduced postprandial glucose in human subjects with T1D, but appeared to be less effective than the subcutaneous formulation. Oral HDV-1 insulin also reduced postprandial glucose in subjects with T2D but did not show a clear dose–response relationship, perhaps because of the precise timing of dosing in relation to meal administration[80].

Extensive efforts continue to be devoted to optimizing oral insulin absorption, through the use of permeation enhancers and protein delivery strategies, and a few examples that have recently demonstrated glucose lowering in preclinical models are highlighted here. Insulin-containing nanoparticles showed improved epithelial uptake, reduced lysosomal targeting of intracellular degradation and enhanced transmucosal passage of insulin relative to native, non-modified insulin alone[81]. Self-assembling 'bubble' carriers composed of diethylene triamine pentaacetic acid (DTPA) dianhydride and sodium bicarbonate can incorporate insulin within the water film of the bubbles. Sodium dodecyl sulfate (SDS) in the bubble carriers enhances the dispersion of insulin molecules, stabilizes the bubble carriers and acts as both a protease inhibitor and a permeation enhancer[82]. An oral insulin preparation formulated with choline and geranate enhanced the paracellular transport of insulin within an ionic liquid formulation while simultaneously reducing enzymatic insulin degradation and decreasing the thickness of the gut mucus layer, to augment exposure of insulin at the surface of the mucosal epithelium[83]. Co-formulation of insulin with deoxycholic acid and chitosan conjugate-coated nano-sized liposomes enables resistance to degradation and enhanced mucosal absorption by targeting the apical sodium-dependent bile acid transporter[84]. Finally, although most oral insulin programmes target the stomach and small intestine as sites of protein absorption, the colon has also been viewed as a favourable region, due to reduced transit times, a more neutral pH, lower levels of degradative enzyme activity and susceptibility to permeation enhancers. Among recently described strategies for colonic peptide targeting, insulin was co-formulated with a number of modified nanoparticles, together with amphipathic chitosan derivatives and a series of CPPs such as Tat, to facilitate transmucosal passage via the transcellular route[85].

Overall, despite the attractiveness of oral insulin delivery, the poor bioavailability of most oral insulin delivery systems, coupled with the high cost of manufacturing recombinant insulin, means that marketing an oral insulin at a competitive price could be challenging, particularly given the intense scrutiny of the rising costs of new insulins[86]. The discontinuation of the clinical development of I338 despite its promising clinical effects illustrates the economic challenges of oral insulin development.

Glucagon-like peptide 1. GLP1 is a gut peptide, originally described as an incretin hormone that potentiates meal-stimulated insulin release (see REF.[87] for a review). Subsequent studies have demonstrated that GLP1 inhibits appetite and promotes weight loss, decelerates gastric emptying and attenuates glucagon secretion — properties collectively useful for the treatment of T2D[87].

Native GLP1 is cleaved at the N terminus by dipeptidyl peptidase-4 (DPP4), necessitating pharmaceutical strategies that circumvent the inactivation of GLP1 (such as DPP4 inhibitors and degradation-resistant peptides) to prolong its half-life. Exenatide (also known as exendin-4), a 39-amino-acid peptide isolated from *Heloderma suspectum* venom, was the first GLP1 receptor agonist approved (in 2005) for the treatment of T2D[88]. Exenatide contains a position 2 glycine, rendering it more resistant to DPP4 than GLP1, and exhibits a longer half-life

in vivo. Exenatide is used clinically as a twice-daily injection, or as a once-weekly long-acting microsphere preparation injected subcutaneously[89]. Multiple short-acting and long-acting GLP1 receptor agonists have been approved for the treatment of T2D, including lixisenatide, a short-acting exenatide analogue suitable for once-daily delivery[90], and liraglutide, an acylated long-acting human GLP1 analogue administered once daily[91], which is also approved at a higher daily dose for the treatment of obesity[92]. Dulaglutide[93], a GLP1–IgGFc fusion protein, and semaglutide[94], an acylated DPP4-resistant human GLP1 peptide analogue[95], are approved for once-weekly dosing.

GLP1 may be well-suited for oral absorption, particularly when engineered for resistance to enzymatic degradation in combination with non-covalent protein binding to enable a prolonged half-life. Unlike insulin, the glucose-dependent mechanisms of GLP1 action minimize the risk of hypoglycaemia. Many strategies have been pursued for the development of orally available GLP1 receptor agonists, most frequently using exenatide (for example, REFS[96–100]) and semaglutide. Efforts with semaglutide have recently culminated in the regulatory approval of an oral formulation developed by Novo Nordisk for the treatment of T2D, based on the largest phase III trial programme conducted to date for an oral peptide. The mechanistic characteristics of this formulation have also been thoroughly studied and reported, so the remainder of this section focuses on this agent.

Semaglutide (M_r = 4,113) was first developed and approved as a once-weekly injectable GLP1 receptor agonist, and this formulation has a circulating half-life of 165 h in humans with T2D[101]. Semaglutide was developed for oral delivery through formulation in a tablet containing the permeation enhancer SNAC[38]. Although SNAC-co-formulated peptides were originally thought to be absorbed in the small intestine[36], more recent research has established the stomach as the major site of oral semaglutide absorption in dogs and humans[38]. Scintigraphic studies showed complete tablet dissolution within the stomach of humans within 60–140 min of ingestion of a tablet containing 10 mg of semaglutide and 300 mg of SNAC, and consistent with the scintigraphic data, pyloric ligation in dogs did not alter the systemic appearance of semaglutide[38]. The bioavailability of oral semaglutide was estimated at 1.22% in dogs[38].

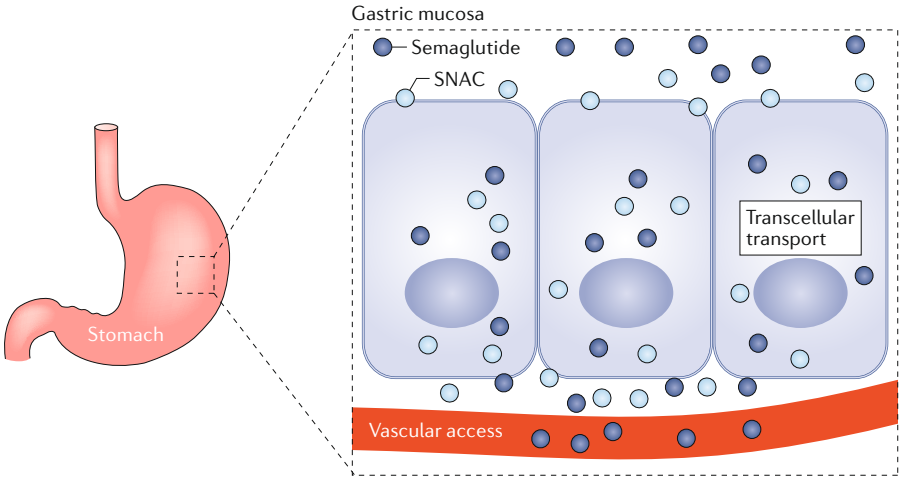

Fig. 3 | **Absorption of oral semaglutide.** The available evidence, derived from studies based on scintigraphy, immunohistochemistry and analysis of drug within the intestinal microcirculation, suggests that oral semaglutide, together with sodium *N*-[8-(2-hydroxybenzoyl)amino] caprylate (SNAC), is predominantly absorbed in the stomach, via the transcellular route[38]. Both SNAC and semaglutide were detected within 30 min of oral ingestion, even in studies in which the stomach was ligated to prevent passage of the SNAC–semaglutide formulation into the intestine[38].

Interestingly, there seems to be a critical range of concentrations for co-formulated SNAC to optimize semaglutide absorption, as 600 mg of SNAC resulted in lower levels of systemic semaglutide than did the 300-mg dose[38]. Furthermore, small differences in either the peptide or the enhancer have substantial effects on absorption efficacy; oral co-administration of SNAC and the closely related GLP1 receptor agonist liraglutide produced negligible circulating levels of liraglutide in rats, and a structural orthoisomer of SNAC, α-SNAC, was markedly less efficient at enhancing semaglutide absorption than SNAC[38]. As the authors highlighted, this indicates that a tailored approach is needed to identify suitable combinations of peptides and permeation enhancers for oral delivery, which may explain the limited success of many efforts in the field in general[38].

Several lines of evidence support a predominant transcellular route for semaglutide absorption (FIG. 3), including the detection of substantial levels of semaglutide within cell monolayers following exposure to SNAC, whereas EDTA (a modulator of tight junction function) did not modify the transport of semaglutide[38]. Semaglutide immunoreactivity was detected in the basal cytoplasm of mucus cells but not in the extracellular spaces between the mucus cells under junctional complexes following oral dosing in rats and dogs[38]. At the molecular level, differential scanning calorimetry revealed physical interactions between SNAC and the cellular lipid membrane. SNAC appeared to shift the proportion of semaglutide molecules forming oligomers to a predominantly monomeric state, which might aid semaglutide absorption. SNAC also probably attenuates the enzymatic digestion of semaglutide by increasing the local gastric pH specifically in the proximity of the semaglutide–SNAC formulation, and absorption of semaglutide was anatomically restricted to the area of the semaglutide–SNAC tablet within the stomach[38].

First-in-human studies have examined single doses (2–20 mg) of oral semaglutide, co-formulated with 150–600 mg SNAC, in healthy human male subjects in the fasting state[102]. Systemic semaglutide exposure was highest when mixed with 300 mg SNAC. Within-subject variability in 24-h AUC semaglutide exposure profiles ranged from 19.7% to 34.9% at steady state, whereas the inter-individual variability in semaglutide levels was considerably greater. The importance of fasting for semaglutide absorption is highlighted by findings of no meaningful detectable systemic semaglutide exposure when the tablet was dosed in the fed state[38]. Oral semaglutide is therefore administered with water in the morning in the fasted state, at least 30 min before breakfast. The extent to which the strict requirement for dosing in the fasting state could limit the adherence to and effectiveness of oral semaglutide is unclear.

Phase II dose-ranging trials of oral semaglutide in subjects with T2D indicated promising effects on blood glucose[103]. Levels of semaglutide were not affected by impaired liver[104] or kidney function[102], and no significant concerns were raised in

studies of drug–drug interactions[105]. The overall safety profile of oral semaglutide was consistent with findings across the GLP1 class. Rates of premature treatment discontinuation, predominantly reflecting gastrointestinal adverse events, were greater with the higher doses of semaglutide, and were >20% with 20-mg and 40-mg doses in phase II studies[103]. The doses of oral semaglutide selected for the phase III programme — 3, 7 and 14 mg once daily — were selected to enable reasonable efficacy without unduly high rates of adverse events.

The efficacy and safety of once-daily oral semaglutide for the treatment of T2D were examined in the Peptide Innovation for Early Diabetes Treatment (PIONEER) programme of eight phase III trials (TABLE 1), including head-to-head studies versus oral agents such as the SGLT2 inhibitor empagliflozin and the DPP4 inhibitor sitagliptin, as well as versus injectable liraglutide[106–112]. The PIONEER trials were carried out in subjects generally representative of the T2D population, with a substantial proportion of study subjects who were overweight or obese, either on metformin therapy alone or metformin plus additional oral antidiabetic agents (TABLE 1). PIONEER 7 examined the efficacy of oral semaglutide in subjects with T2D treated with insulin.

Mean reductions in glycated haemoglobin (HbA1c), from a starting baseline HbA1c generally over 8%, ranged from 0.8% to 1.3% and 1.1% to 1.5%, for the 7-mg and 14-mg doses of oral semaglutide, respectively (TABLE 1), over 26–78 weeks. Weight loss was consistently observed across the programme, ranging from just over 2 kg to 5 kg. The proportion of subjects achieving an HbA1c of <7% on the 14-mg once-daily dose ranged from 59% to 80% (TABLE 1). Nausea and gastrointestinal complaints were the most common adverse events, leading to premature trial discontinuation in ~10–15% of subjects. Collectively, the efficacy and safety of oral semaglutide is consistent with the range of reported responses for injectable GLP1 receptor agonists.

PIONEER 6, the largest and longest trial in the programme (with a 15.9-month median time in the trial), examined the safety of oral semaglutide in 3,183 subjects with T2D at high risk for cardiovascular disease, who were randomized to once-daily oral semaglutide or placebo, with 82% of the subjects being randomized to semaglutide maintained at 14 mg once daily by the end of the study[42]. Fewer cardiovascular events were reported in the subjects randomized to semaglutide (3.8%) than with placebo (4.8%), although the difference was not statistically significant. The rates of cardiovascular death and all-cause mortality were also lower with semaglutide, and both HbA1c reduction (−1.0% versus −0.3%) and body weight loss (−4.2 kg versus 0.8 kg) were greater with semaglutide than with placebo[42]. More subjects discontinued semaglutide than placebo (11.6% versus 6.5%), predominantly due to gastrointestinal adverse events.

Collectively, the results of the PIONEER clinical trial programme (TABLE 1) supported the filing of new drug applications with regulatory authorities. In September 2019, the FDA became the first of these authorities to approve oral semaglutide to improve the control of blood sugar in adult patients with T2D.

Calcitonin. Calcitonin, an amidated 32-amino-acid peptide produced predominantly in the C cells of the thyroid, suppresses bone resorption by osteoclasts. Pharmacological administration of calcitonin lowers blood calcium, but the importance of endogenous calcitonin for physiological control of calcium homeostasis is uncertain[113]. The anti-resorptive properties of calcitonin supported its use as a therapy for acute hypercalcaemia, whereas sustained calcitonin administration has been developed for the treatment of osteoporosis. Salmon calcitonin shares 50% amino acid identity with human calcitonin, yet it is more biologically potent in humans in vivo and was approved as a parenteral injection for the treatment of osteoporosis in 1985, and later as a nasal spray in 1995. The nasal formulation exhibits a bioavailability of 1–3% and was approved for the treatment of osteoporosis on the basis of a 30% reduction of vertebral fractures[114], spurring efforts to develop oral formulations.

Several oral formulations of salmon calcitonin have been investigated in clinical trials[115]. Salmon calcitonin (200 mg formulated in an enteric coating resistant to acid digestion, together with citric acid to enhance protease resistance and paracellular transport) was studied in a phase III trial involving 565 postmenopausal women with osteoporosis[43]. Treatment with this oral formulation resulted in improvements in bone mineral density at the lumbar spine after 48 weeks of treatment that were superior to those obtained with a commercial nasal formulation or placebo, and the oral formulation was safe and as well tolerated as the nasal spray or placebo[43].

Another oral salmon calcitonin agent that has reached phase III trials is a co-formulation with the permeability enhancer 5-CNAC, known as SMC021 (0.8 mg calcitonin plus 200 mg 5-CNAC), which has a reported bioavailability of ~1%[116] and a calculated elimination half-life of 1.5 h[39]. In a placebo-controlled phase III study involving 4,665 postmenopausal women with osteoporosis, treatment with SMC021 resulted in small increases in lumbar spine bone density, but no differences in vertebral fracture rates were detected between the treatment groups[117]. Nausea, gastrointestinal complaints and hot flashes were more commonly reported in subjects treated with SMC021, and more patients discontinued study participation in the SMC021 cohort relative to placebo (17% and 11%, respectively). Unexpectedly, plasma drug levels were lower in this study than were the levels detected in phase I/II testing. The clinical development of SMC021 was halted due to failure to meet the clinical primary end point of fracture reduction[117], perhaps reflecting the suboptimal bioavailability of oral calcitonin over the 3-year study period.

Overall, despite some evidence for clinical anti-resorptive activity of oral calcitonin preparations, the lack of reduction in rates of fracture, concerns about safety and the rapid development of competing anabolic and anti-resorptive agents marketed for osteoporosis[118] have diminished enthusiasm for the commercialization of oral calcitonin formulations.

Parathyroid hormone. PTH is an 84-amino-acid peptide that controls bone resorption and bone formation. The full-length PTH(1–84) molecule is marketed as a once-daily injection for the treatment of hypoparathyroidism[119], whereas the PTH(1–34) molecule, commercially developed in the form of teriperatide, retains bioactivity and is marketed as an anabolic therapy for osteoporosis[120]. Attempts to develop oral human PTH have utilized shorter forms of the molecule, including PTH(1–34) and PTH(1–31), to facilitate transmucosal passage.

EnteraBio has developed an oral formulation of PTH(1–34), which has been tested in phase I trials. Administration of 1.5 mg PTH(1–34) produced a total AUC similar to that achieved following 20 μg of teriperatide injection, with a twofold lower C_{max} for oral PTH (see the Entera Bio press release in the Related links). The permeation enhancer 5-CNAC has also been used to formulate teriperatide for oral delivery. In a pharmacokinetic study of several dose and enhancer combinations in healthy postmenopausal women[121],

Table 1 | **The phase III PIONEER programme for semaglutide**

Patient population	Intervention and comparator	Results on primary end point	Results on secondary end points	Safety findings
PIONEER 1				
Subjects with T2D, previously managed with diet and exercise, prior duration of diabetes of 3.5 years, mean age of 55 years, 51% male, baseline HbA1c of 8%	Oral semaglutide at doses of 3, 7 and 14 mg once daily for 26 weeks versus placebo	Semaglutide produced greater reductions in HbA1c than placebo (0.6%, 0.9% and 1.1% across the three doses) at 26 weeks	Placebo-subtracted differences in body weight with oral semaglutide were 0.1, 0.9 and 2.3 kg	Heart rate and lipase elevations were observed to a greater extent with the higher doses of semaglutide Treatment discontinuation was more common with the 7-mg and 14-mg doses than with placebo, predominantly due to gastrointestinal AEs
PIONEER 2				
Subjects with T2D for ≥90 days, age ≥ 18 years, stable dose of metformin for ≥90 days, HbA1c of 7.0–10.5%	Oral semaglutide initiated at 3 mg once daily, escalated to 7 mg at week 4, and to 14 mg at week 8 versus 25 mg empagliflozin for 26–52 weeks	Semaglutide produced greater reductions in HbA1c (1.4% versus 0.9%) at 26 weeks	Body weight losses were similar (4.2 versus 3.8 kg) at 26 weeks, semaglutide versus empagliflozin, respectively	Rates of common and SAEs were similar; gastrointestinal AEs were more common with semaglutide Trial discontinuation was more common with oral semaglutide than with empagliflozin (10.7% versus 4.4%)
PIONEER 3				
Subjects with T2D, mean age of 58 years, duration of diabetes of 8.6 years, BMI of 32.5, stable dose of metformin ± SU for ≥90 days, entry HbA1c of 7.0–10.5%	Three doses (3, 7 and 14 mg once daily) of oral semaglutide studied, dose escalation at 4-week intervals, versus 100 mg sitagliptin daily	Semaglutide produced a greater reduction in HbA1c than sitagliptin (1.3% for the 14-mg dose versus 0.8%) at 26 weeks	Body weight reductions were greater with oral semaglutide than with sitagliptin: 3.3 versus 0.7 kg at 26 weeks, and 3.5 versus 1.1 kg at 78 weeks, respectively	More subjects on 14 mg semaglutide discontinued trial participation relative to sitagliptin therapy (11.6% versus 5.2%, respectively) Two subjects were positive for anti-semaglutide antibodies at week 26; none at weeks 52–78
PIONEER 4				
Subjects with T2D, mean age of 56 years, 48% female, baseline HbA1c of 7–9.5%, on a background of metformin therapy, with or without a SGLT2 inhibitor	Oral semaglutide (14 mg once daily) versus liraglutide (1.8 mg daily) over 52 weeks	Greater reduction of HbA1c with oral semaglutide than with liraglutide at 52 weeks (1.2% versus 0.9%, respectively)	Weight loss was greater with semaglutide than with liraglutide at 26 and 52 weeks (4.7 versus 3.2 kg and 5.0 versus 3.1 kg, respectively), from a baseline weight of 94 kg	AEs were more common with semaglutide 11% versus 9% versus 4% of participants discontinued treatment early due to AEs, for semaglutide versus liraglutide versus placebo, respectively
PIONEER 5				
Individuals with T2D and reduced eGFR of 30–59 ml/min/1.73 m², duration of diabetes of ~14 years, mean age of 70 years, 52% female, baseline HbA1c of 7–9.5% on a background therapy of ±metformin, ±SU, ±insulin	Oral semaglutide titrated to 14 mg once daily versus placebo for 26 weeks	Semaglutide was superior to placebo with respect to HbA1c reduction (1.1% versus 0.1%) at 26 weeks	Weight loss was greater with semaglutide than with placebo (3.7 versus 1.1 kg)	Gastrointestinal AEs were more common with semaglutide Changes in renal function were not different More subjects discontinued study drug on semaglutide than on placebo (15% versus 5%, respectively)
PIONEER 6				
Subjects with T2D, age >50 years with established cardiovascular or kidney disease, or age >60 years with cardiovascular risk factors	Oral semaglutide titrated to 14 mg once daily, versus placebo and usual care; event driven, median time on drug 15.9 months	Rates of major cardiovascular events were not significantly different for semaglutide versus placebo (hazard ratio, 0.79; 95% confidence interval, 0.57–1.11; $P < 0.001$ for non-inferiority)	Rates of cardiovascular death and all-cause mortality were reduced on semaglutide Reductions in HbA1c (1% versus 0.3%) and body weight (4.2 versus 0.8 kg) were greater with semaglutide than with placebo, respectively	Gastrointestinal AEs leading to discontinuation of study drug were more common with oral semaglutide (11.6% versus 6.5%, respectively)

Table 1 (cont.) | **The phase III PIONEER programme for semaglutide**

Patient population	Intervention and comparator	Results on primary end point	Results on secondary end points	Safety findings
PIONEER 7				
Subjects with T2D, age >18 years, HbA1c of 7.5–9.5%, inadequately controlled on stable daily doses of one or two oral glucose-lowering drugs	Randomization to oral semaglutide with flexible dose adjustments to 3, 7 or 14 mg once daily, or sitagliptin 100 mg once daily over 52 weeks	From a baseline HbA1c of 8.3%, more subjects on semaglutide than on sitagliptin achieved a HbA1c of 7% or less (63% versus 28%, respectively)	Weight loss was greater with oral semaglutide than with sitagliptin (2.9 versus 0.8 kg, respectively)	AEs, predominantly gastrointestinal, were more common with semaglutide
				Drug therapy discontinuation was more common in subjects allocated to semaglutide
PIONEER 8				
Subjects with T2D for ≥90 days, age ≥18 years, HbA1c of 7.0–9.5%, stable dose of either basal, basal-bolus or premixed insulin for ≥90 days ±metformin	3, 7 or 14 mg oral semaglutide once daily versus placebo over 26–52 weeks	Placebo-subtracted HbA1c reduction of 0.6%, 1% and 1.4% were found with 3, 7 and 14 mg of oral semaglutide, respectively, at 26 weeks	Placebo-subtracted reductions in body weight of 1, 2 and 3.3 kg were measured at 52 weeks	Rates of premature study discontinuation were greater with oral semaglutide 14 mg than with placebo (13.3% versus 2.7%, respectively)
			Insulin dose reduction was greater with semaglutide than with placebo	Rates of severe or symptomatic hypoglycaemia were not significantly different (26.5% versus 29.3% for 14 mg of semaglutide versus placebo, respectively)

AEs, adverse events; BMI, body mass index; eGFR, estimated glomerular filtration rate; HbA1c, glycated haemoglobin; SAEs, severe adverse events; SGLT2, sodium–glucose transport protein 2; SU, sulfonylurea; T2D, type 2 diabetes.

the pharmacokinetic profiles of the 2.5-mg and 5-mg oral doses, in combination with 200 mg of 5-CNAC, most closely resembled those obtained with injectable teriperatide, and the bioavailability of oral PTH was estimated to be ~1%[121].

An enteric-coated tablet formulation of recombinant human PTH(1–31)NH_2 containing citric acid has also been tested in humans. In pharmacokinetic studies, the formulation resulted in pulsatile plasma levels of oral PTH(1–31), similar to or greater than those achieved with subcutaneous administration of 20 μg of recombinant PTH(1–34), although greater intra-individual variability in pharmacokinetics was observed with the oral formulation[122]. In an open-label phase II trial in postmenopausal women with osteoporosis, both the C_{max} and the AUC for PTH levels with this oral PTH formulation were similar to or greater than those for teriperatide, but the increase in bone density at the lumbar spine for oral recombinant human PTH(1–31) was lower than that for teriparatide (2.2% compared with 5.1%), and teriperatide resulted in greater and more sustained effects on markers of bone resorption than did oral PTH[123].

The low bioavailability obtained with oral PTH formulations remains a challenge for commercial development. Greater bioavailability (17% following intrajejunal administration) has been reported in a preclinical study in ovariectomized rats, in which enteric microcapsules containing an ionic nanocomplex between PTH(1–34) and lysine-linked deoxycholic acid showed anabolic bone activity[124]. Whether further progress will enable sufficient bioavailability in humans to support the commercial viability of oral PTH formulations remains unclear.

Somatostatin. Octreotide is an octapeptide somatostatin analogue (M_r = 1,019) with high affinity for the somatostatin 2 receptor (SSTR2) and moderate affinity for SSTR5, which has been developed as an injectable therapy (with both short-acting and longer-acting formulations) for the treatment of acromegaly and endocrine tumours[125]. Chiasma has developed an oral formulation of octreotide by encapsulating octreotide with medium-chain free fatty acids and sodium caprylate in order to transiently enhance paracellular permeability through disruption of intestinal tight junction proteins[126]. In pharmacokinetic studies in monkeys and humans, the pharmacokinetic profile of oral octreotide at a 20-mg dose was not substantially different from that obtained with subcutaneous injection of 0.1 mg octreotide[126,127], and oral octreotide administration strongly suppressed secretagogue-stimulated growth hormone secretion in healthy human volunteers[127].

A subsequent phase III trial examined twice-daily oral octreotide in 155 subjects with acromegaly previously controlled with injectable somatostatin receptor agonists[128]. Of the enrolled population, 65% achieved the primary end point, based on insulin-like growth factor 1 (IGF1) and growth hormone levels in the 7-month core treatment period, and 88 of 102 subjects who completed the core treatment (86%) elected to enrol in a 6-month extension[128]. The top-line results of a phase III placebo-controlled trial of oral octreotide for maintenance treatment of adults with acromegaly have recently been reported, with Chiasma announcing that 58% of patients on octreotide capsules maintained their IGF1 response, compared with 19% of the patients on placebo (see the Chiasma press release in the Related links).

Summary and perspectives

Oral peptide delivery is an active and highly promising area, yet formidable challenges remain. Several peptides have been tested in phase III trials, yet only oral desmopressin is widely used in the clinic at present, and it is an exception, as noted above. Insulin has been studied particularly extensively for oral administration. The development of oral insulins is facilitated by the availability of species-specific insulin enzyme-linked immunosorbent assays (ELISAs), and a simple end point, blood glucose, as an immediate readout of pharmacodynamic activity. Nevertheless, progress towards meaningful efficacy in the clinic with oral insulin technologies has been slow and disappointing, with the majority of the vast oral insulin literature reporting on non-clinical data[129]. Moreover, the clinical trial data to date, while demonstrating efficacy, continue to show suboptimal bioavailability, which is a challenge for

cost-effective commercialization of oral insulin formulations[41,129].

While they are exciting from a technological and conceptual viewpoint, oral peptide delivery technologies come with a unique set of safety challenges. Some novel delivery devices may potentially be associated with partial intestinal obstruction, infection or perforation. Controlling for rates of gastrointestinal transit and variable pharmacokinetics arising secondary to unpredictable patterns of drug absorption may be concerning for proteins with narrow therapeutic and safety windows. Understanding the potential pharmacodynamic differences arising from drug absorption via the gut, with more rapid initial hepatic exposure relative to injected therapeutics, may be relevant for drugs such as insulin that exert important actions on both peripheral tissues and the liver[130]. Whether sustained gastrointestinal exposure to high concentrations of active drug substance or permeation enhancers will produce clinically relevant changes in the gut microbiome is not known. Similarly, the extent to which therapeutic proteins delivered via the gastrointestinal tract, often co-formulated with unique chemical entities, will engender unanticipated immune responses requires careful investigation.

The clinical development of oral semaglutide represents the largest phase III programme conducted to date for an oral peptide. While most doses of oral semaglutide assessed in the phase II programme were not as efficacious as once-weekly injected semaglutide[103], oral semaglutide was more effective on HbA1c and weight reduction than was once-daily injectable liraglutide in a head-to-head phase III trial[109]. Several questions surround the ultimate utility and performance of oral semaglutide in the marketplace, including price, long-term tolerability and adherence in the real world, where patients may not always take the medication as instructed on an empty stomach and wait 30 min before meal ingestion. Preliminary indications suggest that despite the much lower bioavailability of oral semaglutide (for which the dose is 14 mg daily, equivalent to 98 mg weekly), it will be priced similarly to injectable semaglutide (for which the dose is 1 mg once weekly). Notably, the once-weekly injectable 1-mg dose appears slightly more effective in reducing HbA1c and body weight, as inferred from phase II studies[103]. Hence, it will be instructive to see how the value proposition for oral semaglutide is perceived and plays out in key markets.

Nevertheless, the phase III data suggest that oral semaglutide is competitive with or better than (in terms of HbA1c and weight reduction) all currently available oral glucose-lowering agents (all of which are less expensive) used to treat T2D. An ongoing larger cardiovascular safety study for oral semaglutide will provide further data on the long-term effects and value of oral semaglutide administration. Competing technologies are also in development, such as allosteric agonists and peptide mimetics with properties suitable for oral drug delivery, as well as small-molecule agonists targeting the GLP1 receptor, such as PF-06882961, which is undergoing clinical assessment in subjects with T2D.

The apparent success of the oral semaglutide development programme is likely to stimulate more interest in new technologies designed to deliver peptide therapeutics and larger proteins via the gastrointestinal tract. To date, the pace of translating the basic science of enteral protein delivery into clinical progress has been slow, with the promise of most novel technologies that have exhibited preclinical efficacy remaining unfulfilled in the clinic. However, the science of protein chemistry, formulation and drug delivery is advancing rapidly and is likely to accelerate, reflecting the opportunities posed by the increasing number of biologics and therapeutic peptides that currently require parenteral administration.

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