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Review article

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Beyond the pancreas: contrasting cardiometabolic actions of GIP and GLP1

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Abstract

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide 1 (GLP1) exhibit incretin activity, meaning that they potentiate glucose-dependent insulin secretion. The emergence of GIP receptor (GIPR)–GLP1 receptor (GLP1R) co-agonists has fostered growing interest in the actions of GIP and GLP1 in metabolically relevant tissues. Here, we update concepts of how these hormones act beyond the pancreas. The actions of GIP and GLP1 on liver, muscle and adipose tissue, in the control of glucose and lipid homeostasis, are discussed in the context of plausible mechanisms of action. Both the GIPR and GLP1R are expressed in the central nervous system, wherein receptor activation produces anorectic effects enabling weight loss. In preclinical studies, GIP and GLP1 reduce atherosclerosis. Furthermore, GIPR and GLP1R are expressed within the heart and immune system, and GLP1R within the kidney, revealing putative mechanisms linking GIP and GLP1R agonism to cardiorenal protection. We interpret the clinical and mechanistic data obtained for different agents that enable weight loss and glucose control for the treatment of obesity and type 2 diabetes mellitus, respectively, by activating or blocking GIPR signalling, including the GIPR–GLP1R co-agonist tirzepatide, as well as the GIPR antagonist–GLP1R agonist AMG-133. Collectively, we update translational concepts of GIP and GLP1 action, while highlighting gaps, areas of uncertainty and controversies meriting ongoing investigation.

Sections

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Key points

- Glucagon-like peptide 1 (GLP1) receptor and glucose-dependent insulinotropic polypeptide (GIP) receptor are widely expressed in multiple organs beyond the pancreas.
- GIP and GLP1 reduce appetite by signalling through their receptors that are expressed in multiple regions of the central nervous system.
- GIP suppresses macrophage-dependent inflammation, whereas GLP1 reduces gut inflammation through its receptor on intraepithelial lymphocytes.
- Both GLP1 and GIP act indirectly on white adipose tissue, whereas GIP directly regulates fat and amino acid metabolism and inflammation within brown adipose tissue.
- GIP and GLP1 are neuroprotective in preclinical models of neurodegenerative disease.
- Current insight into the cardiovascular biology of GIP is limited, whereas GLP1 reduces major adverse cardiovascular events in humans.

Introduction

The incretin concept stems from observations that insulin responses are higher following ingestion of meals or glucose into the gut, relative to the identical isoglycaemic exposure achieved through intravenous glucose administration[1]. The first incretin hormone to be identified, glucose-dependent insulinotropic polypeptide (GIP), was purified from pig gut extracts[2]. Human GIP is a 42 amino acid peptide, and physiological studies in healthy individuals demonstrated that circulating levels of GIP rise briskly following a meal or glucose ingestion. The majority of GIP is synthesized within enteroendocrine K cells in the duodenum and jejunum, although GIP$^+$ endocrine cells are detected more distally in the human small bowel and colon[3]. Mature GIP is cleaved from pre-pro-GIP (a 153 amino acid protein) in the Golgi network and secretory vesicles by prohormone convertase 1 (ref.[4]). Nutrients in the gut lumen, such as glucose, amino acids and fatty acids, stimulate GIP synthesis and secretion.

A second incretin hormone, glucagon-like peptide 1 (GLP1), was identified followed the cloning of the cDNAs and genes that encode proglucagon[5]. Two bioactive isoforms of GLP1 circulate: an amidated 30 amino acid form, GLP1(7–36amide), and a 31 amino acid non-amidated moiety, GLP1(7–37). Although GLP1-producing enteroendocrine L cells are distributed throughout the small and large bowel, the greatest density of L cells is found within the distal small bowel and colon[3]. GLP1 is also synthesized within the brain, predominantly in hindbrain neurons. Pancreatic injury and inflammation induce local islet α-cell GLP1 production; however, the majority of circulating GLP1 is derived from enteroendocrine cells and secreted following nutrient exposure[6].

GIP and GLP1 exert their actions through two distinct yet structurally related class B G protein-coupled receptors (Fig. 1), GIP receptor (GIPR) and GLP1 receptor (GLP1R)[7], first identified in islet β-cells (Box 1). In human islets, GIPR expression is detected in β-cells, α-cells[8], δ-cells[9] and pancreatic polypeptide cells (formerly known as γ-cells). Similarly, GLP1R is expressed in most β-cells, yet low-level GLP1R expression has also been detected in subsets of α-cells and δ-cells[10]. Of note, the two circulating GLP1 isoforms are equipotent at the GLP1R. Detection of incretin receptor expression has been challenging owing to low levels of cellular expression, discordance between sensitivity of detection of RNA and protein and the use of insufficiently validated reagents[11–14]. Multiple studies have demonstrated extrapancreatic expression of both incretin receptors, and their expression patterns are summarized in Fig. 1 (refs.[15–17]).

Importantly, the activation of islet GIPRs augments glucose-stimulated insulin secretion and, to a lesser extent, the glucose-dependent control of glucagon secretion in healthy people and individuals with type 2 diabetes mellitus (T2DM)[18]. Similarly, activation of islet GLP1R signalling stimulates insulin and inhibits glucagon secretion[19]. These physiological actions of GIP and GLP1 are transient, reflecting degradation and inactivation of both hormones by the enzymatic activity of dipeptidyl peptidase 4 (DPP4) and renal clearance. For example, circulating GIP has a short half-life of 5–7 min[20,21]. Inhibition of DPP4 prevents N-terminal inactivation of GIP and GLP1 and potentiates endogenous incretin activity, actions supporting the development of multiple chemically distinct DPP4 inhibitors for the treatment of T2DM[5].

The actions of GIP and GLP1 to reduce glycaemia through glucose-dependent control of insulin secretion provided the initial rationale for exploring the feasibility of incretin-based peptide therapies for T2DM. These are pharmacological incretin receptor agonists that circulate at levels several-fold higher than the native peptides. In individuals with T2DM, the insulinotropic actions of GIP and GIPR agonists are diminished, yet these acute insulinotropic actions are partially restored following brief periods of improved glucose control, as exemplified in studies using insulin[22]. In contrast, GLP1R agonists (GLP1RAs) effectively lower blood levels of glucose in individuals with hyperglycaemia, supporting the development of multiple degradation-resistant GLP1RAs for people with T2DM[23]. GLP1RAs also reduce appetite and body weight, providing the rationale for the development of two GLP1RAs, liraglutide and semaglutide, for weight loss in adolescents and adults with overweight and obesity[24,25]. Importantly, GLP1RAs reduce the rates of major adverse cardiovascular events (MACEs) in people with T2DM, which underscores their utility in the prevention of myocardial infarction, stroke and death in people at risk for cardiovascular events[26]. More recent studies over the past 5 years have examined the potential for GIPR–GLP1R co-agonists to exert favourable metabolic effects beyond those seen with pure GLP1R agonism alone[27].

In this Review, we discuss extrapancreatic actions attributable to GIPR and GLP1R. We contrast physiological functions with actions detected using pharmacological agonists and compare preclinical and clinical data.

Muscle, liver and adipose tissue

GIPR and GLP1R are not expressed in skeletal myocytes, adipocytes or hepatocytes (Fig. 1), yet actions for both GIP and GLP1 are described for muscle, adipose tissue and liver[28]. The indirect effects of GIP and GLP1 on these organs might be related in part to neural signals, circulating effectors or vascular actions, as GLP1 and GIP regulate muscle and adipose tissue blood flow, respectively[29,30].

Skeletal muscle

In the rat soleus muscle, GIP directly increases glucose uptake and GLUT4 expression through a PI3-kinase-dependent pathway. This effect was not blocked with the partial GIPR antagonist Pro(3)-GIP, and *Gipr* mRNA was detected at extremely low levels in skeletal muscle.

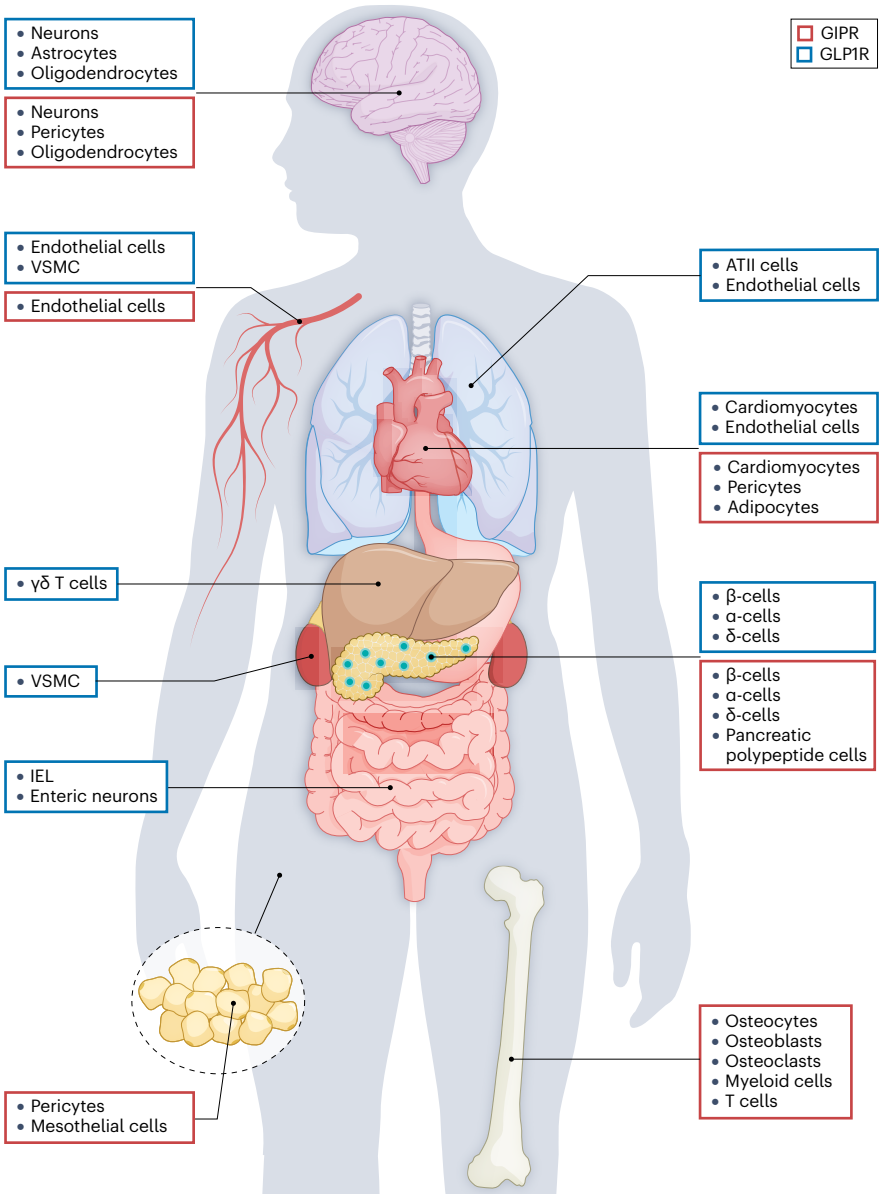

Fig. 1 | Tissue-specific expression of GLP1R and GIPR. The GIPR is expressed within the pancreas, adipose tissue, blood vessels, bone, the heart, haematopoietic and immune cells and the central nervous system[15]. GIPR expression has also been reported in the adrenal and pituitary glands from people with food-induced Cushing syndrome or acromegaly[181,182] (not shown in figure). The GLP1R is expressed in the pancreas, heart, kidneys, gut, blood vessels, liver, lungs and the central and peripheral nervous systems[19,28]. ATII, alveolar epithelial type II; GIPR, glucose-dependent insulinotropic polypeptide receptor; GLP1R, glucagon-like peptide 1 receptor; IEL, intraepithelial lymphocyte; VSMC, vascular smooth muscle cell.

Hence, the signalling mechanisms that link GIP to augmentation of muscle glucose transport remain unknown[31].

GLP1 regulates muscle blood flow in vivo, but whether GLP1 directly stimulates muscle blood flow is less clear and depends on the experimental context. Local skeletal muscle perfusion of GLP1 (10 μM) for 80 min in the gastrocnemius muscle of healthy volunteers had no effect on muscle blood flow[32]. In contrast, systemic delivery of native GLP1 acutely enhanced muscle microvascular blood flow in rats and humans; however, indirect contributions from hormones such as insulin or bioactive GLP1 degradation products cannot be excluded[29,33,34]. Whether degradation-resistant clinically approved GLP1RAs directly increase muscle blood flow, independent of insulin, in people with T2DM and/or obesity remains unclear.

Liver

Although GIPR expression is not identified in the liver, GIP indirectly modulates hepatic lipid metabolism via unclear mechanisms. A 6-day infusion of native GIP in lean men with type 1 diabetes mellitus increased

Box 1

Incretin receptors

Structurally related glucose-dependent insulinotropic polypeptide receptor (GIPR) and glucagon-like peptide 1 receptor (GLP1R) mediate the physiological and pharmacological actions of glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1, respectively[7]. Notably, both peptides are degraded into smaller fragments by dipeptidyl peptidase 4 and other proteases, including neutral endopeptidases. These fragments exert unique biological activities transduced through signalling pathways independent of the known canonical incretin receptors.

Splice variants of *GIPR* differentially regulate receptor activity, including a carboxy-terminally truncated isoform that acts in a dominant negative manner to diminish receptor signalling in β-cells[183]. Multiple *GIPR* variants have also been identified in human visceral adipose tissue and subcutaneous adipose tissue, the majority of which remain incompletely characterized[184]. Emerging evidence published in a preprint publication suggests a loss of function phenotype for the *GIPR* variant (E354Q) linked to reductions in BMI, body weight, weight and hip circumference, levels of insulin and bone formation. These findings correlated with reduced receptor activation and decreased β-arrestin recruitment in cells ex vivo[185]. Preliminary data published in a preprint paper suggest that GIPR interacts with a subset of receptor activity modifying proteins for modulation of GIPR activity (cellular localization and the temporal profile of signalling)[186]. However, the importance of receptor activity modifying proteins for the extrapancreatic actions of gastric inhibitory polypeptide is not known.

A different preprint communication reported more than a hundred different missense variants in *GLP1R*, many with altered signalling properties[187]; however, whether these *GLP1R* variants modify the physiological actions of endogenous glucagon-like peptide 1 or the pharmacological responses to GLP1R agonists (GLP1RAs) in various tissues is not definitively established. In addition, a fourth preprint paper indicates that the *GLP1R* variant rs6923761G>A (Gly168Ser) was associated with a modestly greater reduction in HbA_{1c} in response to GLP1RA (0.9 mmol/mol), whereas several low-frequency variants in *ARRB1* (encoding β-arrestin) were also associated with a much greater 2.7 mmol/mol HbA_{1c} reduction in people with type 2 diabetes mellitus, relative to individuals treated with a non-GLP1RA glucose-lowering agent[188].

hepatic lipid content, with no effect on the white adipose tissue (WAT) transcriptome, circulating lipid species, the plasma proteome or circulating biomarkers of inflammation[35]. In obese mice, antagonism or genetic knockout of GIPR or ablation of gut *Gip* expression improves metabolic outcomes, reduces hepatic lipid accumulation and lowers expression of markers of liver inflammation[36–38]. Interestingly, the GIP–GLP1 co-agonist tirzepatide (discussed later) also exerts beneficial liver effects in mice and humans, but its hepatic actions beyond those indirectly associated with weight loss and indirect effects on insulin and glucagon secretion are unknown.

GLP1RAs reduce hepatic steatosis and liver inflammation in animals and humans, and semaglutide is being studied in phase III trials for the therapy of non-alcoholic steatohepatitis (NASH)[39]. Hepatocytes do not express the canonical GLP1R[11,40], but a small subset of intrahepatic γδ T cells express a functional GLP1R and might contribute to some of the anti-inflammatory actions of GLP1RA in mouse liver[41]. However, these $GLP1R^+$ intrahepatic T cells have not yet been identified in human studies.

Adipose tissue

The expression and activity of GIPR in adipose tissue cell types is somewhat controversial (Fig. 2). GIP acutely increases abdominal adipose tissue blood flow and enhances adipose tissue triglyceride clearance in humans studied under hyperinsulinaemic–hyperglycaemic clamp conditions, designed to mimic post-prandial physiology[42]. The augmentation of adipose tissue blood flow by meals or GIP is blunted in people with obesity and partially restored by weight loss induced by caloric restriction or bariatric surgery[30,43]. Direct lipogenic actions of GIP have been demonstrated using primary adipocyte cell cultures and adipocyte-like cell lines[44]. In human adipocytes cultured ex vivo, GIP promotes activation of lipoprotein lipase, an enzyme involved in triglyceride hydrolysis and uptake of circulating free fatty acids[45]. GIP also promotes insulin sensitization, glucose uptake, de novo lipogenesis as well as lipolysis in isolated rat adipocyte-like cells[46] (Fig. 2). However, in rodents and non-human primates, loss of GIP function via pharmacological antagonism or whole-body genetic knockout reduces accumulation of adipose mass and is protective against diet-induced obesity[36,38,47,48].

Although *GIPR* expression is detected in cultured human or mouse adipocyte-like cells ex vivo, detection of GIPR expression within adipocytes studied in vivo is more difficult. One study attributed the overlapping beneficial metabolic phenotypes of enhanced versus blocked GIPR signalling to a loss of adipocyte GIPR activity that occurs with sustained GIPR activation. However, in this study, adipocyte GIPR expression was not demonstrated using mouse adipose tissue in vivo, only in adipocyte-like cells studied ex vivo[49]. In another study, *Fab4*-Cre was used to inactivate GIPR in mouse adipose tissue, resulting in high-fat diet (HFD)-fed mice with a phenotype of reduced insulin resistance and decreased hepatic steatosis[50]. Surprisingly, no major changes in adipose tissue mass or function were detected in these mice, despite exhibiting 90% knockdown of adipose *Gipr* expression. In contrast, findings from a third study showed substantial reduction of both adipose and brain *Gipr* expression using *Fab4*-Cre to inactivate *Gipr*, yet failed to detect reduction of adipose tissue *Gipr* expression using multiple lines of *Adipoq*-Cre mice[51]. Moreover, single nucleus RNA-sequencing (RNA-seq) studies did not localize *GIPR* expression to adipocytes within adipose tissue; rather, *GIPR* expression was detected in mouse and human pericytes and human mesothelial cells[51].

The canonical GIPR is detected in brown adipose tissue (BAT), predominantly in pericytes. GIP but not GLP1 directly regulates a thermogenic gene expression profile in mouse BAT[52] and upregulates lipid, amino acid and glucose catabolic processes in obese insulin-resistant mice[53,54]. Mice with *Gipr*-deficient BAT ($Gipr^{BAT-/-}$) exhibit increased body temperatures after an acute cold challenge; however, body weight, energy expenditure, glucose metabolism and lipid metabolism are not different in HFD-fed $Gipr^{BAT-/-}$ versus $Gipr^{BAT+/+}$ mice[52]. Hence, the loss of BAT GIPR does not recapitulate the resistance to obesity phenotype of whole-body $Gipr^{-/-}$ mice. The putative importance of GIPR signalling in human BAT has not been ascertained.

GLP1-regulated central nervous system (CNS) pathways might indirectly drive BAT activation and augment thermogenesis and energy

expenditure in mice[55] but not in humans[56] (Fig. 2). GLP1RA administration augments glucose uptake and substrate flux in muscle, adipose tissue and liver, probably through indirect mechanisms, reflecting improved blood flow, enhanced insulin secretion, neural communication and changes in energy substrate flux reflecting weight loss over time.

Potential actions in bone

GIP acutely stimulates bone formation and inhibits bone resorption in animals[57,58] and humans, independent of changes in insulin or glucose[59]. Furthermore, genetic variation within the human *GIPR* associates with differences in BMD and fracture risk[60] (Fig. 1). Notably, the anti-resorptive actions of GIP in people with type 1 diabetes mellitus were not sustained after 6 days of continuous GIP infusion[35]. In contrast, GLP1 regulates calcitonin secretion in mice and rats but not in humans[61,62]. In addition, GLP1RAs have had little impact on BMD or fracture rates, even in older adults with T2DM who initiate GLP1RA therapy[63]. Hence, it is interesting to assess whether the use of GIP-based therapies (co-agonists or antagonists) alters clinically meaningful musculoskeletal endpoints in large outcome trials.

Haematopoietic and immune systems

GIPR

GIPR is expressed by myeloid lineages, including monocytes and macrophages and some bone marrow T cells[64,65] (Fig. 3). Whole-body *Gipr* deletion in mice impairs haematopoiesis, evidenced by decreased numbers of bone marrow-derived myeloid-progenitor cells, circulating monocytes and macrophages[64]. In HFD-fed mice, myeloid cell-specific deletion of *Gipr* leads to upregulated S100A8-dependent pro-inflammatory signalling in adipose tissue, impaired insulin action and reduced energy expenditure[65,66]. Whole-body ablation of *Gipr* in mice, or more selectively in haematopoietic lineages, revealed a role for GIPR signalling in the modulation of the expression of genes encoding Toll-like receptors and Notch proteins within the bone marrow[65].

Administration of GIP reduces inflammation in adipose tissue. For example, HFD-fed mice treated with the long-acting GIP analogue [d-Ala(2)] GIP for 8 weeks have increased lipid storage, yet reduced expression of pro-inflammatory cytokines and chemokines and decreased macrophage accumulation in WAT, associated with reduced insulin resistance, compared with control HFD-fed mice not treated with a GIP analogue[67]. Similarly, widespread transgenic *Gip* expression under the control of the metallothionein promoter decreased adipose tissue mass, reduced macrophage infiltration and attenuated the expression of *Ccl2*, *Serpin1*, *Il4ra* and *Ikbkb*, genes encoding pro-inflammatory proteins, in WAT of HFD-fed mice[68]. Taken together, these experiments imply that GIP agonism decreases body weight, reduces inflammation and indirectly enables healthy adipose tissue expansion, which might have the consequence of preventing excess ectopic lipid accumulation and inflammation associated with obesity.

On the contrary, GIPR agonism without meaningful weight loss might also promote adipose tissue inflammation. For example, intraperitoneal administration of GIP twice daily for 1–4 weeks increased *Ccl2* and *Il6* mRNA transcripts in multiple adipose depots of *db/db* mice, with increased WAT macrophage infiltration in retroperitoneal adipose tissue[69]. Infusion of GIP (4 h) into otherwise healthy individuals with obesity (achieving circulating levels of GIP of ~120 pM) induced a pro-inflammatory gene signature in subcutaneous WAT, with induction of mRNAs encoding the chemokines CCL2 and CCL8, and IL-6, together with increased circulating levels of CCL2 (ref.[70]). Similar findings were detected following infusion of GIP into healthy male individuals with

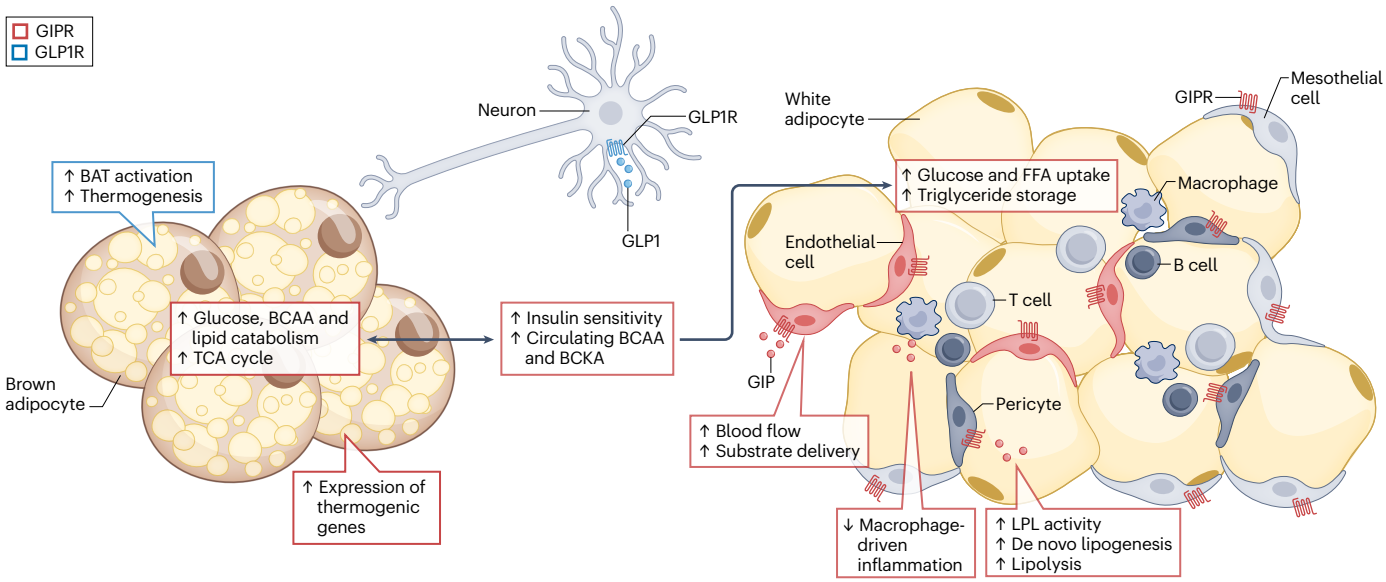

Fig. 2 | The role of GIP and GLP1 in white adipose tissue-related and brown adipose tissue-related metabolic processes. GIP receptor (GIPR), but not GLP1 receptor (GLP1R), is expressed within adipose tissue. In white adipose tissue (WAT), GIPR is expressed in endothelial cells, macrophages, pericytes and mesothelial cells. GIP stimulates blood flow to WAT, lipoprotein lipase (LPL) activity, insulin sensitization, glucose and free fatty acid uptake, de novo lipogenesis as well as lipolysis. GIP also regulates WAT macrophage-dependent inflammation. In brown adipose tissue (BAT), GIP regulates thermogenesis-related genes and upregulates lipid, amino acid and glucose catabolic processes. Conversely, GLP1 indirectly promotes BAT activation and thermogenesis via central nervous system pathways in mice and rats. GIP–GIPR-affected processes are highlighted in red and GLP1–GLPR1-affected processes are highlighted in blue. BCAA, branched-chain amino acid; BCKA, branched-chain keto acid; FFA, free fatty acid; GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like peptide 1; TCA, tricarboxylic acid.

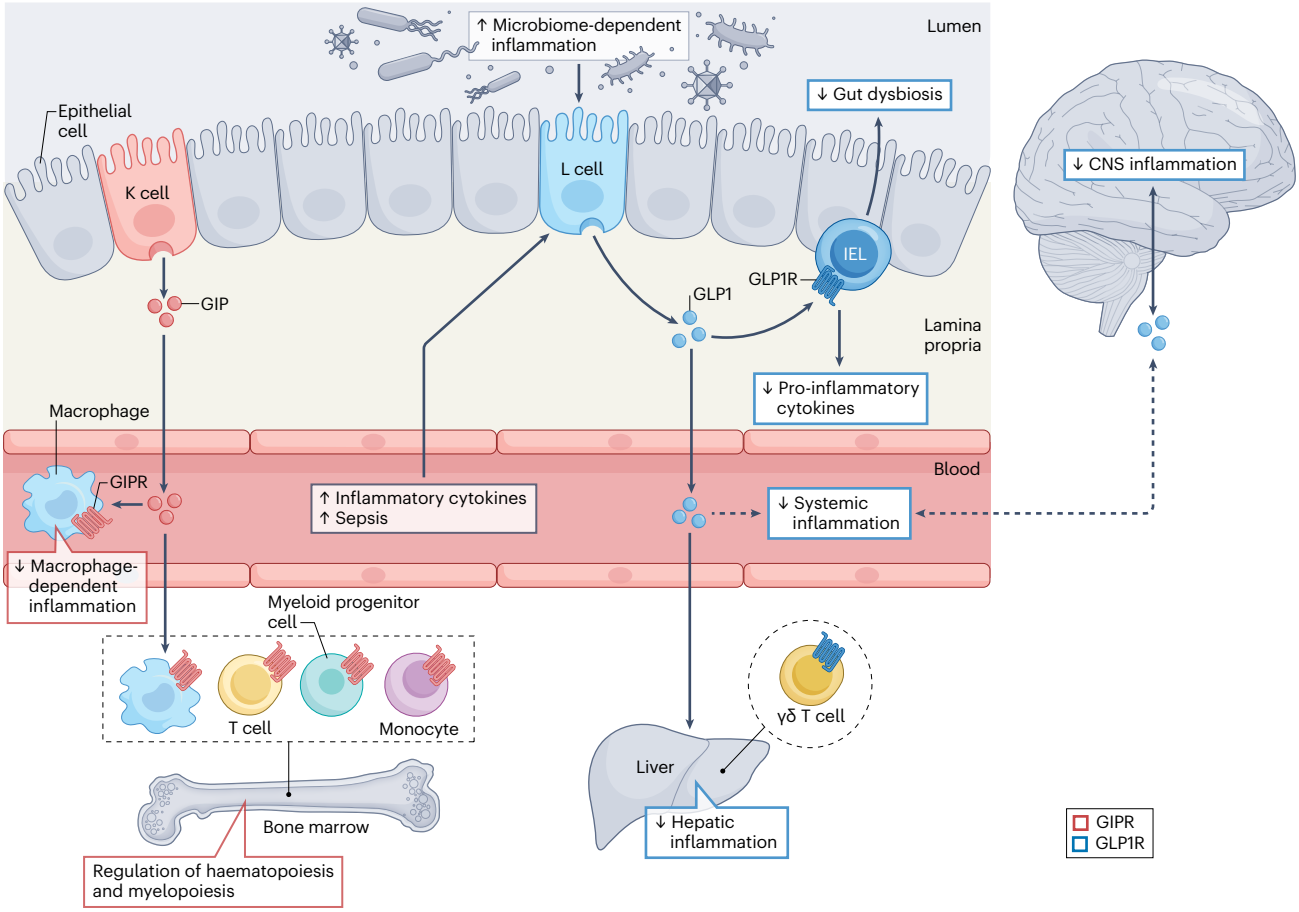

Fig. 3 | The actions of GIP and GLP1 on the haematopoietic and immune systems. GIP receptor (GIPR), but not GLP1 receptor (GLP1R), is expressed by cells of the myeloid lineage, including macrophages, monocytes and myeloid-progenitor cells, as well as some T cells. Bone-marrow-expressed GIPR regulates myelopoiesis as well as systemic and adipose tissue-specific inflammation. In the gut, the cellular localization of GIPR remains unknown, whereas GLP1R is detectable within intraepithelial lymphocytes (IELs) and enteric neurons. Sepsis, elevated circulating inflammatory cytokines or microbiome-dependent inflammation, stimulates the L cells within the gut to release GLP1, which acts on its corresponding receptor on IELs to blunt the inflammatory response. Activation of IEL GLP1R also inhibits gut dysbiosis. GLP1 acts pharmacologically to reduce inflammation in multiple tissues, such as the central nervous system (CNS), that do not contain GLP1R$^+$ immune cells. GIP–GIPR-affected processes are highlighted in red and GLP1–GLPR1-affected processes are highlighted in blue. GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like peptide 1.

a BMI >28 kg/m^2 under hyperinsulinaemic–euglycaemic clamp conditions, findings not replicated by hyperinsulinaemia alone. In addition, GIP directly increased levels of *Il5* and *Il6* mRNA in mouse brown adipocyte-like cells cultured ex vivo, whereas acute administration of GIP increased plasma levels of IL-6 in wild-type mice[52]. Conversely, reduction of *Gipr* in mice tissues, achieved through use of *Fabp4*-Cre, reduced adipose expression of IL-6 and cytokine signalling-3 (SOCS3)[50]. As GIPR expression is not detected within mouse or human adipocytes in vivo, these disparate consequences of gain versus loss of GIPR signalling on adipose tissue might reflect indirect actions on vascular and immune cells, as well as differences in experimental methods and endpoints.

GLP1R

Expression of GLP1R within the immune system (Figs. 1,3) is detected within intestinal intraepithelial lymphocytes (IELs)[71] and at much lower levels in a subset of hepatic γδ T cells[41]. Inflammation, microbial metabolites and cytokines induce the secretion of GLP1, but not GIP, in mice and humans[72–75]. Indeed, the L cell functions as a sensor of pathogens and inflammation, integrating signals from microbial metabolites and cell wall products to augment GLP1 secretion in response to infection or sterile injury. Notably, circulating levels of GLP1 rise rapidly after myocardial infarction in animals and humans and correlate with the extent of myocardial injury[76]. In addition, plasma levels of GLP1 correlate with the severity of illness and survival in people hospitalized for critical illness, including sepsis[77,78]. Although GLP1 serves as a biomarker reflecting the severity of inflammation, little evidence is available to support a therapeutic role for pharmacological administration of GLP1RAs to improve outcomes in people with acute myocardial infarction or sepsis.

GLP1RAs reduce systemic and tissue inflammation in mice, often independent of changes in body weight[79,80], through poorly understood mechanisms[23]. Although some studies link GLP1R activation to reduction of macrophage-dependent inflammation[81], localizing canonical

GLP1R expression within macrophages is difficult[11]. GLP1RAs also exert anti-inflammatory actions in people with T2DM, both acutely[82] and following sustained administration, independent of weight loss[83]. Mechanistically, very low levels of *Glp1r* mRNA transcripts can be detected in immune cells within mouse lymph nodes or spleen[84]; however, the major site of immune cell GLP1R expression is intestinal IELs[71]. Direct activation of GLP1R on mouse IELs reduces inflammation, whereas failure to develop a normal population of GLP1R^{+} IELs in *Itgb7*$^{-/-}$ mice results in increased GLP1 secretion, improved metabolism and reduction of experimental atherosclerosis[85]. Interestingly, these phenotypes are not recapitulated in mice with T cell deficiency or more selective ablation of *Glp1r* in T cells[80].

IEL GLP1R also regulates microbiome-dependent inflammation through calibration of GLP1 secretion. Notably, *Glp1r*$^{-/-}$ mice exhibit gut dysbiosis[71] and the actions of GLP1 on modulating the gut microbiota independent of changes in glucose or body weight are mediated in part through IEL GLP1R[80]. The IEL GLP1R is also important in T cell-dependent gut inflammation, but this cellular site of immune cell GLP1R signalling is not essential for the anti-inflammatory actions of GLP1RAs in the context of systemic inflammation induced by lipopolysaccharide in mice[80].

Microorganisms such as *Akkermansia muciniphilia* secrete one or more proteins that directly enhance GLP1 secretion in mice[86]. Microbial metabolites, including bile acids, activate the gut–brain axis after experimental bariatric surgery in mice via enhanced GLP1 secretion[87]. Furthermore, in HFD-fed mice, gut microbial dysbiosis is associated with acquired GLP1 resistance, findings characterized by elevated levels of portal vein GLP1 and reduced GLP1R expression within the enteric nervous system[88]. Notably, the normal circadian control of GLP1 secretion is disrupted in germ-free or antibiotic-treated mice[89]. The extent of hypothalamic inflammation is also reduced in germ-free versus conventionally housed HFD-fed mice, with elevated circulating levels of GLP1 detected in germ-free mice, findings that are dependent on the presence of functional GLP1Rs in astrocytes[90]. Hence, both local and systemic enhancement of microbiota-dependent inflammation is regulated in part by GLP1R signalling. Whether treatment with GLP1RAs consistently modulates the composition and diversity of gut microbiota in people with T2DM, independent of changes in glycaemia or body weight, remains uncertain[91].

GLP1 also reduces hepatic inflammation in preclinical studies and in humans with NASH, actions that might be partly independent of weight loss[41,92,93]. Clinical studies examining the actions of liraglutide and semaglutide in people with NASH demonstrated reduction of inflammation without progression of fibrosis, as inferred from liver biopsies taken before and after therapy with GLP1RAs[39,94]. Hepatocytes do not express the canonical GLP1R[28]. However, in mouse liver, GLP1R expression has been localized to hepatic endothelial cells, as well as a subset of γδ T cells[41]. Genetic disruption of *Glp1r* within haematopoietic and endothelial cells partially attenuated the anti-inflammatory actions of semaglutide in the liver of HFD-fed mice[41]. The actions of once weekly semaglutide are being studied in 1,200 people with NASH in a phase III trial (NCT04822181).

Renal biology

The circulating levels of endogenous GIP and GLP1 are dependent on renal metabolism and are increased in people with chronic kidney disease (CKD) and renal failure[95,96]. GIP action on the kidney has not been carefully studied, in keeping with a lack of detectable renal GIPR expression[15].

GLP1R is expressed in the kidney, localized to a subset of vascular smooth muscle cells (VSMCs) within afferent arterioles[97,98] (Fig. 4). GLP1 acutely enhances sodium and water excretion and reduces albumin excretion in both rodent and human studies, in the presence or absence of diabetes mellitus[99–101]; however, the natriuretic actions of liraglutide are diminished with sustained use in people with T2DM[101]. GLP1RA are renoprotective in mice and rats with experimental kidney disease[102]. The anti-inflammatory, anti-fibrotic and renoprotective actions of GLP1RAs in mice with experimental nephrotoxic nephritis[103] have been attributed to GLP1R expression within T cells, enabling direct inhibition of T cell proliferation[104].

The use of GLP1RAs in people with T2DM has been associated with rare reports of reversible acute kidney injury, mostly detected early in the course of therapy. This serious adverse effect is secondary to nausea, vomiting and dehydration, leading to hypovolaemia, often in people with pre-existing kidney disease[105]. Although short-acting exendin-based GLP1RAs are not utilized for glucose control in people with T2DM and CKD, the human GLP1RAs liraglutide, dulaglutide and semaglutide are effective and safe to use for the reduction of glucose in people with T2DM and reduced estimated glomerular filtration rate (eGFR), including those with CKD[106].

GLP1RAs reduce blood pressure in people with T2DM or obesity and hypertension, which might indirectly contribute to renoprotection. In large prospective studies of individuals with T2DM, GLP1RAs have not yet been shown to meaningfully reduce renal outcomes, beyond reduction of albumin excretion[107]. However, post hoc analyses of individuals with T2DM treated with liraglutide, semaglutide and dulaglutide in the LEADER, SUSTAIN-6 and REWIND cardiovascular safety trials, respectively, reveal a modest reduction in the rate of decline in eGFR, most notable in people with eGFR <60 ml/min/1.73 m^2 (refs.[108,109]). Furthermore, analysis of real-world data in Scandinavia demonstrated reduced rates of serious renal events (consisting of renal replacement therapy, renal death and hospitalization for renal events) in people treated with GLP1RAs from 2010 to 2016 (ref.[110]). The long-term renal safety and renoprotective potential of semaglutide are being assessed in a clinical study of people with T2DM, reduced eGFR and elevated urinary albumin excretion (NCT03819153).

Cardiovascular actions

GIPR

GIPR is expressed within some blood vessels (endothelial cells) and in the mouse and human heart[111] (Fig. 4). Whether increased plasma levels of GIP are associated with atherosclerosis in humans is controversial[112], as distinct variants within *GIPR* differentially associated with increased levels of GIP and the risk of heart disease[113]. Loss of whole-body *Gipr* and selective loss of cardiomyocyte *Gipr* expression are cardioprotective in mice[12], whereas GIPR agonism reduces atherosclerosis in sensitized animal models[114]. Conversely, studies of *Gipr*$^{-/-}$ mice reveal increased aortic inflammation and enhanced atherosclerosis[115]. Human genetics associates glucose-lowering alleles of *GIP* and *GIPR* with reduced BMI, triglycerides and lower rates of heart failure[116].

Mechanisms linking the action of GIP to cardiovascular outcomes remain unclear. Studies of animal models of atherosclerosis or hypertensive cardiomyopathy or myocardial infarction describe a role for GIP in the suppression of macrophage-driven inflammation and foam cell formation[114,117,118]. GIP also reduces VSMC proliferation, arterial remodelling and nitric oxide synthesis[119], cardiomyocyte hypertrophy, cardiac fibrosis and myocardial fatty acid metabolism

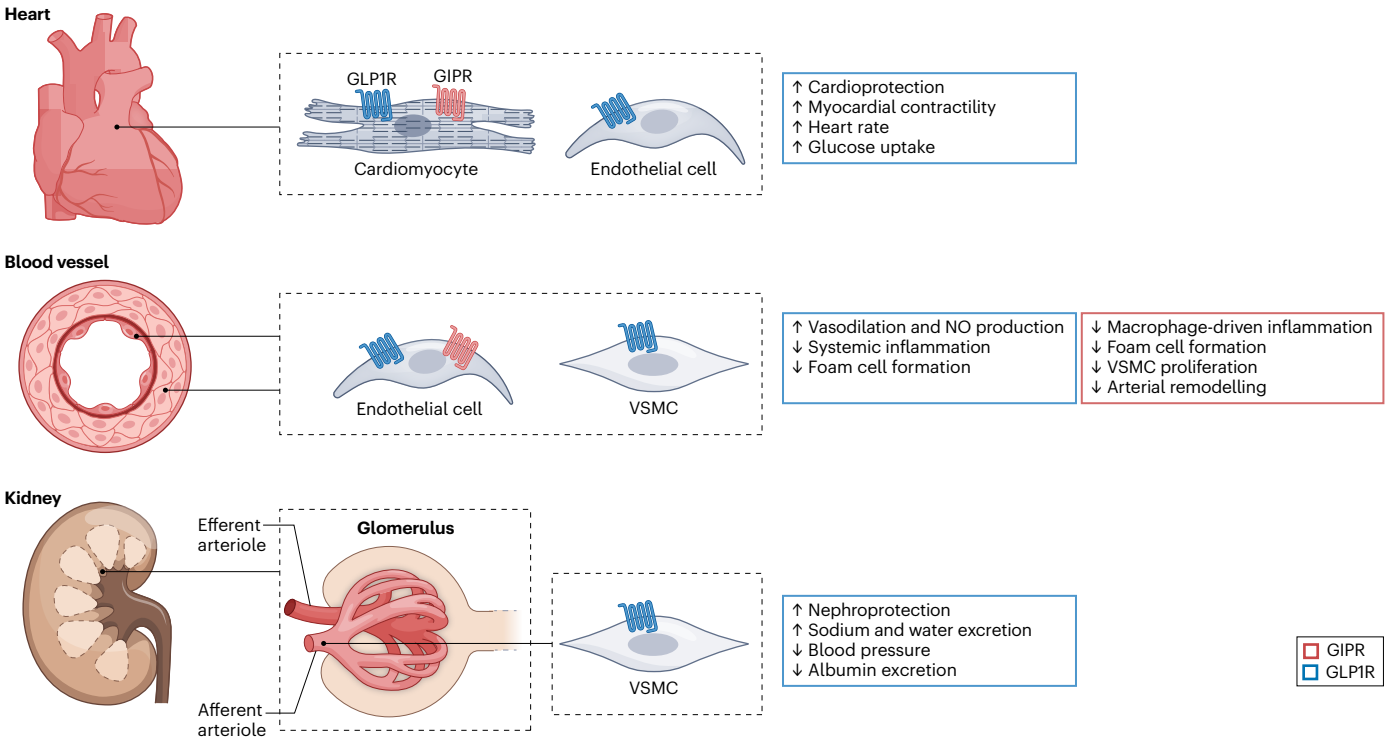

Fig. 4 | The actions of GIP and GLP1 in the cardiovascular and renal systems. GLP1 has direct and indirect effects in the reduction of cardiovascular disease risk via its cardioprotective, nephroprotective and anti-inflammatory actions. GLP1 receptor (GLP1R) is expressed at low levels in cardiomyocytes and endothelial cells in the heart. Within the cardiovascular and renal systems, the GLP1R has been localized to a subset of cardiomyocytes, endothelial cells and vascular smooth muscle cells (VSMCs). GIP receptor (GIPR) is expressed in cardiomyocytes in the heart and in some endothelial cells within blood vessels. GIP might have an indirect role in atherosclerosis, via the regulation of macrophage-driven inflammation and foam cell formation, VSMC proliferation and arterial remodelling. GIP–GIPR-affected processes are highlighted in red and GLP1–GLPR1-affected processes are highlighted in blue. GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like peptide 1; NO, nitric oxide.

and storage[12,120]. Nevertheless, whether these actions are direct or indirect, and conserved in humans, is not currently known.

GLP1RAs

GLP1RAs reduce blood levels of glucose, blood pressure, body weight, systemic inflammation and post-prandial lipaemia in multiple animal species and humans, actions consistent with a favourable reduction in cardiovascular risks[121]. GLP1 might also reduce platelet aggregation; however, expression of the canonical GLP1R in platelets remains uncertain[122]. Of note, GLP1R activation is linked with reduction of gut lipoprotein secretion, but the mechanisms are not well understood. Enterocytes do not express GLP1R, and genetic ablation of neural GLP1R pathways does not attenuate the GLP1R-dependent reduction of gut chylomicron secretion in mice[123]. Within the cardiovascular system, GLP1Rs are expressed in subsets of endothelial cells, VSMCs and in cardiac atria and ventricles[28,111] (Fig. 4). Preclinical studies demonstrate that administration of GLP1RAs reduces infarct size, while improving ventricular function and survival in mice with experimental myocardial infarction[124]. Similarly, liraglutide improved ventricular function independent of changes in body weight in mice with HFD-associated cardiomyopathy[79]. The GLP1R is expressed in some human cardiomyocytes and mouse endocardial endothelial cells[111,125]. Genetic targeting of endothelial cell, but not cardiomyocyte *Glp1r* expression, attenuated the cardioprotective actions of liraglutide in mice with ischaemic cardiac injury[125,126].

GLP1RAs also reduce plaque burden and aortic inflammation in mice with experimental atherosclerosis[127,128]. Notwithstanding the sparse expression of the canonical GLP1R in vascular cells[41], the vasoprotective and blood pressure-lowering actions of GLP1RAs were abolished in mice with endothelial cell-selective inactivation of *Glp1r* that were treated with angiotensin II[129]. GLP1R is expressed in endothelial cells within the mouse aorta; however, semaglutide treatment attenuated the development of atherosclerosis in mice with genetic ablation of GLP1R in endothelial and haematopoietic cells[41].

Cardiovascular safety studies of long-acting GLP1RAs in people with T2DM have demonstrated reductions in MACE, principally myocardial infarction stroke and death from cardiovascular causes[26]. Hospitalization for heart failure is also reduced by ~11% with GLP1RAs in cardiovascular outcome trials[26]. The cardiovascular benefits of GLP1RAs become evident within 12–24 months of trial initiation[130]. In contrast to findings with sodium–glucose cotransporter 2 inhibitors, GLP1RAs consistently reduce the rates of stroke in people with T2DM[26]. Real-world and clinical trial data suggest that the cardiovascular benefits of sodium–glucose cotransporter 2 inhibitor and GLP1RAs are additive in people with T2DM[131,132]. Whether GLP1RAs reduce the rates of MACE and heart failure in people with obesity in the absence of

T2DM is not yet known and will be informed in part by the results of the SELECT trial[133].

Mechanistically, administration of liraglutide for 26 weeks to people with T2DM had no effect on vascular inflammation assessed by [^{18}F]-fluorodeoxyglucose PET scanning of the carotid arteries and aorta[134]. However, a substudy of the same individuals demonstrated attenuated coronary artery inflammation as visualized by [^{64}Cu]Cu-DOTATATE uptake and PET scanning, findings that correlated with reduction of C-reactive protein[135]. Administration of exenatide once weekly for 18 months to people with T2DM reduced body weight and HbA_{1c}, but had no effect on carotid plaque volume or composition[136]. Similarly, liraglutide (1.8 mg daily for 26 weeks) had minimal effect on inflammation-related gene expression in white blood cells from people with T2DM[137]. Hence, whether GLP1RAs meaningfully reduce vascular and/or systemic inflammation and atherosclerosis in people with T2DM, independent of changes in glucose or body weight, remains uncertain and will require longer periods of study.

Neural actions

CNS expression of GIP, GLP1 and their receptors

GIP is detected by immunohistochemistry in multiple regions of the rat brain, including the hippocampus (dentate gyrus), olfactory bulb, brainstem, cerebral cortex and cerebellum[138,139], whereas GIPR expression has been detected in the cortex, hippocampus, olfactory bulb and hypothalamus[15] (Fig. 5). A GIPR-YFP reporter mouse revealed GIPR$^+$ transcriptional activity within the paraventricular, dorsomedial and arcuate nuclei of the hypothalamus[140]. Single-cell RNA-seq analysis of mouse hypothalamic neurons identified co-expression of *Gipr* with mRNA transcripts encoding appetite and energy expenditure regulatory neuropeptides, including somatostatin, vasopressin and cocaine-regulated and amphetamine-regulated transcript protein, as well as neurohormone receptors for GABA, glutamate, histamine, acetylcholine, serotonin, somatostatin, calcitonin, ghrelin and cholecystokinin[140].

Notably, GIPR$^+$ cells within the hypothalamus are not exclusive to neurons. Single-cell RNA-seq analysis of isolated GLP1R$^+$ and GIPR$^+$ hypothalamic cells from reporter mice revealed that GLP1R$^+$ cells are evenly distributed among neuronal and non-neuronal hypothalamic cell types, including VSMCs. In contrast, the majority of GIPR$^+$ cells were non-neuronal including oligodendrocytes and vascular cells, predominantly pericytes[141]. Transcriptional interrogation of GLP1R$^+$ cells in the brains of mice and non-human primates identified multiple noradrenergic GLP1R$^+$ neuronal subtypes. Furthermore, exogenous GLP1RA administration regulated gene expression profiles within cells of the area postrema and in calcitonin receptor$^+$ cells in the nucleus of the solitary tract[142]. Specifically, semaglutide upregulated genes (including *Bdnf* and *Mc4r*) and pathways in glutamatergic GLP1R$^+$ neurons and suppressed gene transcription in glial cells. In humans and mice, a subset of hypothalamic cells within the arcuate nucleus and the dorsal medial hypothalamus and other brain regions co-express GIPR and GLP1R[140] (Fig. 5).

GIP, food intake and body weight

Both gain and loss of GIPR signalling reduce body weight. HFD-fed whole-body *Gipr*$^{-/-}$ mice exhibit less weight gain and improved insulin sensitivity compared with wild-type mice, although this phenotype takes several months to emerge[36,47]. Plasma levels of glucocorticoids are decreased in *Gipr*$^{-/-}$ mice; however, resistance to weight gain is maintained despite physiological glucocorticoid replacement[143].

Multiple studies demonstrate that selective GIPR antagonism attenuates weight gain. Sustained systemic GIPR blockade reduced body weight gain and food intake in mice and non-human primates, findings independent of the β-cell GIPR as shown in *Gipr*$^{\beta cell-/-}$ mice[38]. Similarly, whole-body GIPR antagonism with a GIPR antibody (Gipg013) reduces food intake and attenuates weight gain in HFD-fed mice, without changes in energy expenditure. However, systemic GIPR antagonism using once weekly peripheral administration of Gipg013 was less effective at reducing body weight in mice with established diet-induced weight gain[48]. Central intracerebroventricular administration of the Gipg013 antagonist also reduces body weight and adiposity in mice with diet-induced obesity, findings associated with decreased hypothalamic levels of SOCS3, an inhibitor of leptin signalling[144].

A key role for the CNS in the resistance to obesity phenotype observed in *Gipr*$^{-/-}$ mice was further established in studies using *Nestin*-Cre to selectively ablate GIPR signalling in the CNS[145]. Elimination of *Gipr* in the CNS was sufficient to recapitulate the resistance to diet-induced obesity phenotype seen in whole-body deficient mice. However, HFD-fed CNS-specific *Gipr*$^{-/-}$ mice showed an attenuated anorectic and weight-loss response to the GIPR agonist acyl-GIP, whether given by peripheral injection or intracerebroventricular administration[145]. Hence, both gain and loss of GIPR signalling reduces body weight through mechanisms that require the CNS GIPR.

GLP1, appetite, gut motility and body weight

GLP1 action in the brain engages multiple circuits linked to stress responses, aversion, anorexia, hypothalamic pituitary function, neuroinflammation and neuroprotection[23,146] (Fig. 5). GLP1RAs also inhibit gastric and small bowel motility in multiple species, actions mediated through CNS-dependent and myenteric neuron-dependent mechanisms in mice[123,147]. Notably, the actions of GLP1 to inhibit gastric emptying are subject to tachyphylaxis within a few hours of sustained GLP1R signalling[148]. Physiologically, autonomic GLP1R$^+$ neurons are required for the basal control of gastric emptying in mice[123]. By contrast, GLP1R$^+$ neurons within the *Wnt1* expression domain and vagal neurons are critical for the inhibition of gastric emptying in response to pharmacological GLP1R agonism in mice[123] and humans[149].

Humans treated with GLP1RAs exhibit reduced food intake and weight loss, supporting the approval of liraglutide and semaglutide for weight loss in people with overweight or obesity[24,25,146]. Although nausea and vomiting might be dose-limiting and prompt discontinuation of GLP1RAs in some individuals, these adverse events exhibit rapid tachyphylaxis and are less common with gradual versus more rapid dose up-titration. Multiple distributed GLP1R$^+$ neurons within at least 10 different regions of the mouse and rat CNS, including the hypothalamus and hindbrain, transduce pharmacological signals from GLP1RAs and couple them to the behavioural outcome of reduction of food intake[150]. Consistent with these findings, selective targeting of GLP1R$^+$ neurons in the hypothalamus or hindbrain does not completely attenuate the weight-reducing actions of GLP1RAs in mice or rats[151,152].

Neuroprotection

A role for GIP in the control of hippocampal neurogenesis has been identified in the rat and mouse brain. GIP induces the proliferation of rat hippocampal granule cell layer neural progenitor cells, whereas *Gipr*$^{-/-}$ mice show evidence of reduced cell proliferation in the granule cell layer of the adult hippocampus[138]. GIP is also neuroprotective in experimental animal models of dysregulated metabolism and neurodegenerative diseases such as Alzheimer disease, Parkinson disease and Huntington disease[153]. GIP might elicit its neuroprotective effects in mice in part through the promotion of neurogenesis[154].

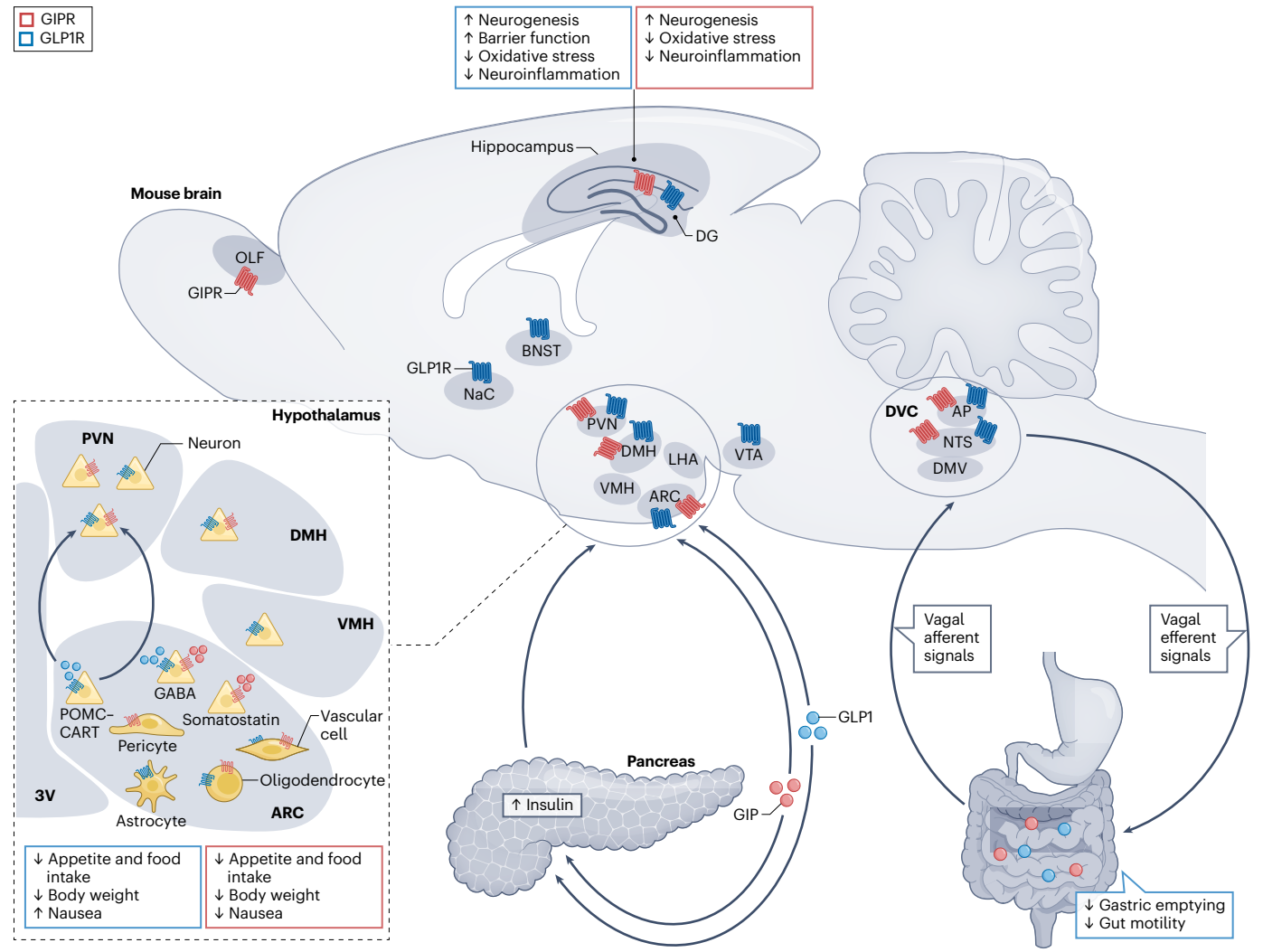

Fig. 5 | The role of GIP and GLP1 in central energy regulation and neuroprotection. Within the mouse and human brain, expression of GLP1 receptor (GLP1R) and GIP receptor (GIPR) is localized to the hypothalamus, including the arcuate nucleus (ARC), dorsomedial hypothalamus (DMH) and paraventricular nucleus (PVN). Neurons within the hypothalamus have distinct GIPR and GLP1R expression profiles, with some neurons co-expressing both receptors. The receptors are also expressed in the dentate gyrus (DG) of the hippocampus, nucleus tractus solitarius (NTS) and area postrema (AP). GLP1R is also expressed within the ventral tegmental area (VTA), bed nucleus of the stria terminalis (BNST) and nucleus accumbens (NaC). GLP1R and GIPR have been identified in neurons and oligodendrocytes. In addition, GIPR is expressed in pericytes and GLP1R is expressed in astrocytes. GIP and GLP1 elicit neuroprotective effects in the brain. GLP1 also decreases gastric emptying and motility via vagal afferent and efferent signals to the dorsal vagal complex (DVC). GIP–GIPR-affected processes are highlighted in red and GLP1–GLPR1-affected processes are highlighted in blue. 3V, third ventricle; DMV, dorsal motor nucleus of the vagus; GIP, glucose-dependent insulinotropic polypeptide; GLP1, glucagon-like peptide 1; LHA, lateral hypothalamic area; OLF, olfactory bulb; POMC–CART, proopiomelanocortin–cocaine-regulated and amphetamine-regulated transcript; PVN, paraventricular nucleus; VMH, ventromedial hypothalamus; VTA, ventral tegmental area.

The GIP analogue d[Ala2]-GIP administered for 4 weeks in mice that had been fed a HFD for 5 months improved the recognition index and attenuated the impairment in long-term potentiation, in the absence of weight loss[155]. Treatment of mice for 5 weeks with once daily d[Ala2]-GIP attenuated the development of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson disease, in association with reduced loss of dopamine neurons and decreased α-synuclein expression in the substantia nigra pars compacta[156]. Similarly, a 14-day course of d[Ala2]-GIP reduced the appearance of neurobehavioural deficits and preserved levels of striatal monoamines in rats with quinolinic acid-induced Huntington disease-like pathology[157]. Multiple studies have demonstrated that GIP analogues such as d[Ala2]-GIP reduce the burden of amyloid plaque and decrease levels of oxidative stress and the extent of neuroinflammation, while enhancing long-term potentiation in experimental models of Alzheimer disease, including APP/PS1 mice[158]. In contrast, much less information is available on the impact of GIPR blockade in the context of experimental neurodegeneration.

Substantial preclinical data demonstrate that GLP1RAs are neuroprotective in experimental models of stroke, Parkinson disease or Alzheimer disease[23,159]. Several small, randomized trials examined the effects of twice daily or once weekly exenatide versus conventional therapy for 12 months in people with moderate Parkinson disease without T2DM and demonstrated improved motor activity scores that persisted following discontinuation of exenatide[160,161]. Longer and larger ongoing studies are examining the efficacy of once weekly exenatide over 96 weeks in 200 individuals with mild-to-moderate Parkinson disease[162].

The neuroprotective actions demonstrated in preclinical studies might reflect both direct and indirect actions of GLP1RAs, encompassing enhanced neuronal proliferation and survival, reduced oxidative stress, decreased neuroinflammation and improved barrier function[159]. Moreover, HFD-fed germ-free mice exhibit increased levels of circulating GLP1 and reduced hypothalamic inflammation, findings that are mediated by the astrocyte GLP1R[90]. Human studies of 12 weeks of liraglutide administration revealed improved brain connectivity in individuals with normal cognitive function but a history of subjective cognitive complaints[163]. These experiments have prompted examination of whether the use of GLP1RAs might prevent cognitive decline in people with or without T2DM. Analysis of people with T2DM treated with dulaglutide in the REWIND trial revealed a 14% reduction in cognitive impairment, as assessed through serial testing at baseline and during follow-up[164]. Similarly, reported rates of dementia were decreased in people randomized to GLP1RA therapy in the LEADER, SUSTAIN-6 and PIONEER-6 cardiovascular outcome trials, as well as in real-world population registry data among individuals with T2DM treated with a GLP1RA for at least 5 years[165]. Two randomized controlled trials, EVOKE (NCT04777396) and EVOKE+ (NCT04777409, the latter enrolling at least 20% of the study population with established cerebrovascular disease), have been initiated to examine the therapeutic effect of oral semaglutide on the change in a dementia rating scale over 2 years in people at high risk for developing Alzheimer disease.

Combinatorial incretin therapy

Multi-agonist peptide therapies

The success of GLP1RAs for the treatment of metabolic disorders reflects the preservation of the glucoregulatory and anorectic actions of GLP1RAs in people with T2DM and obesity. Although GIP is physiologically important for incretin action in animals and humans[166], the insulin stimulatory actions of GIP are diminished in the setting of experimental and clinical diabetes mellitus[22]. Nevertheless, administration of insulin for 4 weeks to lower HbA_{1c} improved the β-cell response to GIP in people with T2DM. This finding implies that the hyperglycaemia-associated reduction in GIP responsivity is partially reversible[22]. Moreover, the remarkable reductions in HbA_{1c} and weight loss achieved with the GIPR–GLP1R co-agonist tirzepatide in people with T2DM[167] have rekindled enthusiasm for understanding the biology and mechanisms that underlie the effectiveness of combined GIPR and GLP1R agonism[27]. Tirzepatide achieved double-digit percent total body weight loss in the SURPASS trials in people with T2DM[146] and more than 20% placebo-subtracted body weight loss in people with obesity treated with 15 mg once weekly in the SURMOUNT-1 trial[168]. Studies in mice demonstrate that weight loss achieved with tirzepatide is predominantly mediated through the GLP1R[53]; however, tirzepatide is a suboptimal agonist at the mouse GIPR[54]. Tirzepatide therapy also reduces hepatic steatosis[169], and clinical trials of tirzepatide in people with metabolic liver disease are underway. Whether the reduction of hepatic steatosis observed with tirzepatide is independent of weight loss in humans, perhaps reflecting neural or immune mechanisms and interorgan communication, has not yet been conclusively addressed in preclinical or clinical studies.

Although GIPR agonism does promote reduction of food intake and weight loss in preclinical studies, the magnitude of the effect is modest[145,170,171]. Furthermore, GIPR agonism does not seem to be additive as modelled using combined chemogenetic activation of $GIPR^+$ and $GLP1R^+$ neurons[140]. Similarly, in men with overweight or obesity, co-infusion of GIP and GLP1 over 4 h did not additively reduce food intake during a test meal, beyond that achieved with GLP1 infusion alone[172]. Mechanistically, central administration of a GLP1R–GIPR dual agonist in mice had a greater effect on reduction of food intake relative to GLP1R agonism alone. Additionally, the superior efficacy of the co-agonist was lost in mice with elimination of the GIPR in the CNS[145]. Studies of tirzepatide action in HFD-fed mice reveal that tirzepatide-induced weight loss requires the GLP1R, whereas tirzepatide enhanced insulin sensitivity through GLP1R-independent pathways, possibly reflecting contributions from activation of BAT[53]. Interpretation of these findings requires acknowledgement that tirzepatide is a suboptimal agonist at the mouse GIPR, relative to the human GIPR[54].

Combinatorial therapy with GIPR–GLP1R dual agonists and GIPR–GLP1R–glucagon receptor tri-agonists has also shown superior neuroprotective effects relative to GLP1RA monotherapy in rodent models of Alzheimer disease and Parkinson disease[173,174]. However, little attempt was made to tease out the relative mechanistic contributions of each receptor in these multi-agonist studies. Moreover, no information is available on the neuroprotective potential of GIPR–GLP1R dual agonists in humans. Intriguingly GIPR agonism reduces GLP1RA-induced aversive behaviours in mice and rats and emesis in musk shrews[175]. Additionally, genetic and pharmacological activation of $GIPR^+$ neurons suppressed nausea-related behaviours through area postrema inhibitory circuits in mice[176], raising the possibility that GIPR agonism might improve the tolerability of a co-administered GLP1RA (Fig. 5).

Clinical evidence from phase II studies shows that treatment with tirzepatide reduces biomarkers of cardiovascular disease, including chitinase-3-like protein 1, intercellular adhesion molecule 1 and high-sensitivity C-reactive protein, in people with T2DM[177]. Whether tirzepatide reduces inflammation independent of changes in glucose or body weight has not been studied. The cardiovascular safety of tirzepatide across the SURPASS programme was reported in a pre-specified meta-analysis, revealing hazard ratios of 0.8 for four-point MACE, 0.9 for cardiovascular death and 0.8 for all-cause mortality[178]. A dedicated cardiovascular outcome trial, SURPASS CVOT (NCT04255433), is examining the safety of tirzepatide relative to dulaglutide in people with T2DM and established cardiovascular disease, with an expected completion date in the fourth quarter of 2024.

GIPR antagonism

Notwithstanding the enthusiasm for GIPR–GLP1R co-agonism, extensive data show that *Gipr*$^{-/-}$ mice are resistant to diet-induced obesity[179], findings mediated in part through enhanced energy expenditure[47]. Of note, HFD-fed mice and non-human primates treated with GIPR antagonists, alone or in combination with GLP1RAs, resist weight gain and exhibit improved metabolic control[38]. Consistent with these findings, a GIPR antagonist–GLP1RA single antibody produced robust reductions in body weight and adipose tissue mass in obese mice and monkeys, achieved predominantly through a reduction in food intake[180]. Moreover, a humanized GIPR antagonist–GLP1RA antibody (AMG-133) is being studied in clinical trials in people with obesity (NCT04478708).

Conclusions

Although GLP1 action has been studied extensively in the clinic, mechanisms underlying the GLP1-dependent control of inflammation, atherosclerosis, blood pressure, gut chylomicron secretion and hepatocyte lipid metabolism remain incompletely understood. Furthermore, mechanisms might differ in animals versus humans. The interest in GIP biology has been re-energized by the clinical development of tirzepatide. Interpreting the actions of GIPR multi-agonists in humans can be challenging owing to limited investigational exposure of humans to GIP and GIPR agonists. GIP has direct actions on bone, β-cells, α-cells and the immune system (Fig. 6); however, data informing the sustained actions of GIP in humans, with or without cardiometabolic disorders, are limited. Moreover, accurate ascertainment of expression of the canonical GIPR in cells and organs remains challenging owing to low levels of expression and the limitations of available reagents[13]. Whether GIPR agonism produces substantial weight loss in humans remains uncertain. Studies in mice link GIPR signalling using mono-agonists or tirzepatide to reduced food intake and robust activation of BAT[52,53]. However, analysis of human BAT activity and its potential contribution to insulin sensitivity or weight loss in humans treated with GIPR agonists or co-agonists is limited, and such studies would be of interest.

New unimolecular agents, such as AMG-133 that combines GLP1R agonism with GIPR antagonism, have been developed, which have the potential to induce effective weight loss with only once monthly

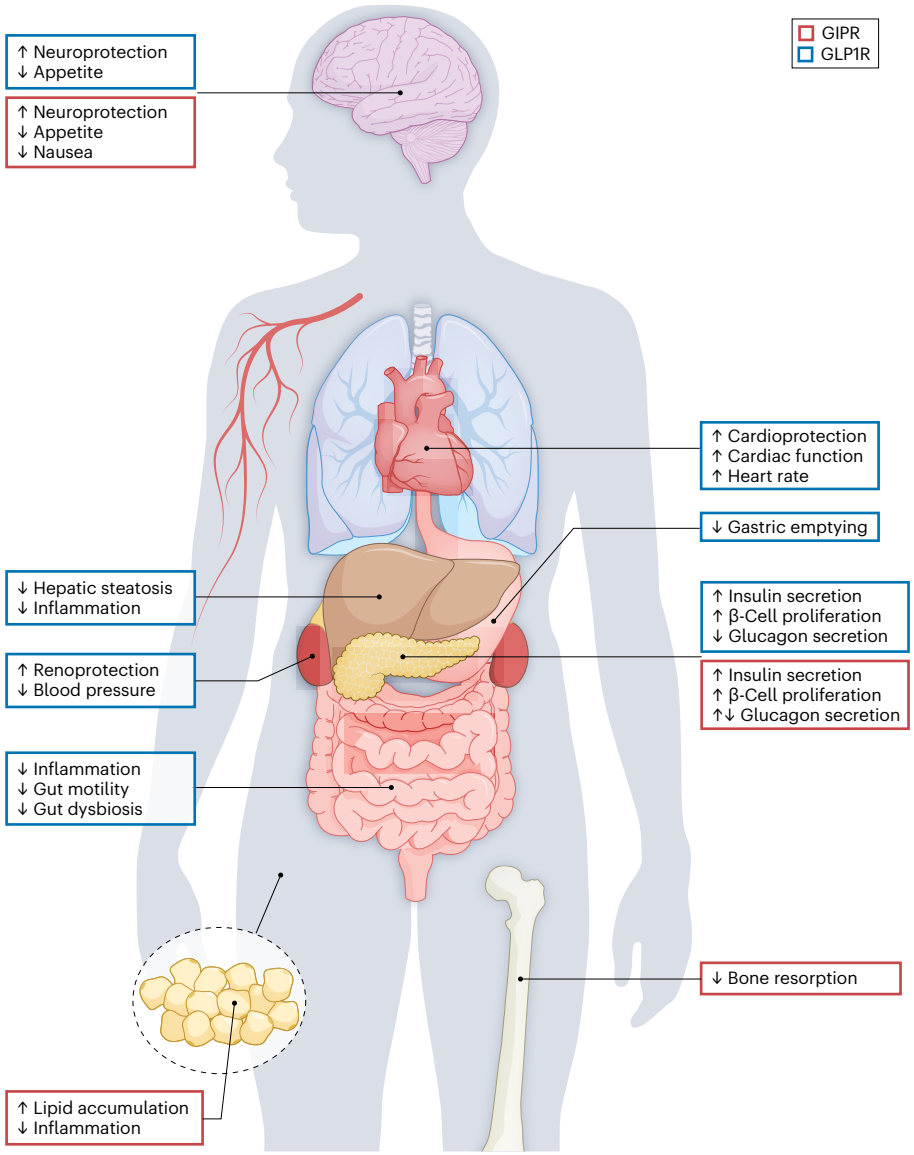

Fig. 6 | A simplified summary of the biological actions of GIP and GLP1. Beyond the incretin actions of GLP1 and GIP to control insulin secretion and glycaemia, GLP1 and, to a lesser extent, GIP, exert multiple extrapancreatic actions. These actions affect inflammation, cardiovascular and renal function, gastrointestinal motility, bone and mineral homeostasis and neurological function. Of note, these actions are relevant for the treatment of cardiometabolic disorders. GIP–GIP receptor (GIPR)-affected processes are highlighted in red and GLP1–GLP1 receptor (GLP1R)-affected processes are highlighted in blue. GIP, glucose-dependent insulinotropic polypeptide; GLP1R, glucagon-like peptide 1.

dosing (NCT04478708). How can one reconcile the competing data supporting GIPR agonism versus GIPR blockade as effective strategies for weight loss? Evidence supports GIP acting directly on GIPR in the CNS to inhibit food intake and perhaps also indirectly by enhancing the anorectic effect of GLP1. Chronic GIPR agonism might theoretically elicit some of its anti-obesity effects in the brain and the periphery via receptor desensitization, a hypothesis that is consistent with the favourable metabolic phenotype described for *Gipr*$^{-/-}$ mice[36]; however, this theory has not yet been convincingly established. Although evidence for desensitization of the GIPR has been reported in adipose tissue[49], limited evidence exists so far for GIPR desensitization in the brain. GIPR agonism might also enhance the tolerability and hence the efficacy of GLP1RAs by lowering the associated nausea and aversive response to GLP1RA treatment, potentially improving patient compliance[175,176].

Ultimately, the utilization of GIP–GLP1-based co-agonists for the chronic treatment of metabolic disorders will be influenced to a large extent by the assessment of safety. Ongoing cardiovascular outcome trials in people with T2DM or obesity will be extremely informative for assessing the benefits of these therapies beyond traditional metabolic endpoints, such as HbA_{1c} reduction and weight loss. Given the therapeutic trajectory for GLP1RAs, one might also expect the emergence of orally available co-agonists, as well as the interrogation of GIP-based therapies in people with NASH, heart failure with preserved ejection fraction or neurodegenerative disorders. The available data strongly suggest that understanding the long-term benefits and mechanisms of action for GIP–GLP1 combinations will be of substantial interest and clinical importance to the metabolism community in the years to come.

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References

1. McIntyre, N., Holdsworth, C. D. & Turner, D. S. Intestinal factors in the control of insulin secretion. *J. Clin. Endocrinol. Metab.* **25**, 1317–1324 (1965).
2. Brown, J. C. & Dryburgh, J. R. A gastric inhibitory polypeptide. II. The complete amino acid sequence. *Can. J. Biochem.* **49**, 867–872 (1971).
3. Jorsal, T. et al. Enteroendocrine K and L cells in healthy and type 2 diabetic individuals. *Diabetologia* **61**, 284–294 (2018).
4. Ugleholdt, R. et al. Prohormone convertase 1/3 is essential for processing of the glucose-dependent insulinotropic polypeptide precursor. *J. Biol. Chem.* **281**, 11050–11057 (2006).
5. Drucker, D. J., Habener, J. F. & Holst, J. J. Discovery, characterization, and clinical development of the glucagon-like peptides. *J. Clin. Invest.* **127**, 4217–4227 (2017).
6. Song, Y. et al. Gut-proglucagon-derived peptides are essential for regulating glucose homeostasis in mice. *Cell Metab.* **30**, 976–986.e3 (2019).
7. Mayo, K. E. et al. International union of pharmacology. XXXV. The glucagon receptor family. *Pharmacol. Rev.* **55**, 167–194 (2003).
8. El, K. et al. GIP mediates the incretin effect and glucose tolerance by dual actions on alpha cells and beta cells. *Sci. Adv.* **7**, eabf1948 (2021).
9. Segerstolpe, A. et al. Single-cell transcriptome profiling of human pancreatic islets in health and type 2 diabetes. *Cell Metab.* **24**, 593–607 (2016).
10. Ast, J. et al. Super-resolution microscopy compatible fluorescent probes reveal endogenous glucagon-like peptide-1 receptor distribution and dynamics. *Nat. Commun.* **11**, 467 (2020).
11. Panjwani, N. et al. GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE−/− mice. *Endocrinology* **154**, 127–139 (2013).
12. Ussher, J. R. et al. Inactivation of the glucose-dependent insulinotropic polypeptide receptor improves outcomes following experimental myocardial infarction. *Cell Metab.* **27**, 450–460 (2018).
13. Ast, J., Broichhagen, J. & Hodson, D. J. Reagents and models for detecting endogenous GLP1R and GIPR. *eBioMedicine* **74**, 103739 (2021).
14. Gray, S. M. et al. Discordance between GLP-1R gene and protein expression in mouse pancreatic islet cells. *J. Biol. Chem.* **295**, 11529–11541 (2020).
15. Usdin, T. B., Mezey, E., Button, D. C., Brownstein, M. J. & Bonner, T. I. Gastric inhibitory polypeptide receptor, a member of the secretin-vasoactive intestinal peptide receptor family, is widely distributed in peripheral organs and the brain. *Endocrinology* **133**, 2861–2870 (1993).
16. Campos, R. V., Lee, Y. C. & Drucker, D. J. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. *Endocrinology* **134**, 2156–2164 (1994).
17. Dunphy, J. L., Taylor, R. G. & Fuller, P. J. Tissue distribution of rat glucagon receptor and GLP-1 receptor gene expression. *Mol. Cell Endocrinol.* **141**, 179–186 (1998).
18. Christensen, M. B., Calanna, S., Holst, J. J., Vilsboll, T. & Knop, F. K. Glucose-dependent insulinotropic polypeptide: blood glucose stabilizing effects in patients with type 2 diabetes. *J. Clin. Endocrinol. Metab.* **99**, E418–E426 (2014).
19. Campbell, J. E. & Drucker, D. J. Pharmacology physiology and mechanisms of incretin hormone action. *Cell Metab.* **17**, 819–837 (2013).
20. Mentlein, R., Gallwitz, B. & Schmidt, W. E. Dipeptidyl-peptidase IV hydrolyses gastric inhibitory polypeptide, glucagon-like peptide-1(7–36)amide, peptide histidine methionine and is responsible for their degradation in human serum. *Eur. J. Biochem.* **214**, 829–835 (1993).
21. Kieffer, T. J., McIntosh, C. H. & Pederson, R. A. Degradation of glucose-dependent insulinotropic polypeptide and truncated glucagon-like peptide 1 in vitro and in vivo by dipeptidyl peptidase IV. *Endocrinology* **136**, 3585–3596 (1995).
22. Hojberg, P. V. et al. Four weeks of near-normalisation of blood glucose improves the insulin response to glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with type 2 diabetes. *Diabetologia* **52**, 199–207 (2009).
23. Drucker, D. J. Mechanisms of action and therapeutic application of glucagon-like peptide-1. *Cell Metab.* **27**, 740–756 (2018).
24. Pi-Sunyer, X. et al. A randomized, controlled trial of 3.0 mg of liraglutide in weight management. *N. Engl. J. Med.* **373**, 11–22 (2015).
25. Wilding, J. P. H. et al. Once-weekly semaglutide in adults with overweight or obesity. *N. Engl. J. Med.* **384**, 989 (2021).
26. Sattar, N. et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of randomised trials. *Lancet Diabetes Endocrinol.* **9**, 653–662 (2021).
27. Baggio, L. L. & Drucker, D. J. Glucagon-like peptide-1 receptor co-agonists for treating metabolic disease. *Mol. Metab.* **46**, 101090 (2021).
28. McLean, B. A. et al. Revisiting the complexity of GLP-1 action from sites of synthesis to receptor activation. *Endocr. Rev.* **42**, 101–132 (2021).
29. Wang, N. et al. Vasodilatory actions of glucagon-like peptide 1 are preserved in skeletal and cardiac muscle microvasculature but not in conduit artery in obese humans with vascular insulin resistance. *Diabetes Care* **43**, 634–642 (2020).
30. Asmar, M. et al. Glucose-dependent insulinotropic polypeptide has impaired effect on abdominal, subcutaneous adipose tissue metabolism in obese subjects. *Int. J. Obes.* **38**, 259–265 (2014).
31. Snook, L. A., Nelson, E. M., Dyck, D. J., Wright, D. C. & Holloway, G. P. Glucose-dependent insulinotropic polypeptide directly induces glucose transport in rat skeletal muscle. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **309**, R295–R303 (2015).
32. Bertin, E., Arner, P., Bolinder, J. & Hagstrom-Toft, E. Action of glucagon and glucagon-like peptide-1-(7–36) amide on lipolysis in human subcutaneous adipose tissue and skeletal muscle in vivo. *J. Clin. Endocrinol. Metab.* **86**, 1229–1234 (2001).
33. Chai, W. et al. Glucagon-like peptide 1 recruits microvasculature and increases glucose use in muscle via a nitric oxide-dependent mechanism. *Diabetes* **61**, 888–896 (2012).
34. Subaran, S. C. et al. GLP-1 at physiological concentrations recruits skeletal and cardiac muscle microvasculature in healthy humans. *Clin. Sci.* **127**, 163–170 (2014).
35. Heimburger, S. M. N. et al. GIP affects hepatic fat and brown adipose tissue thermogenesis, but not white adipose tissue transcriptome in T1D. *J. Clin. Endocrinol. Metab.* https://doi.org/10.1210/clinem/dgac542 (2022).
36. Miyawaki, K. et al. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat. Med.* **8**, 738–742 (2002).
37. Althage, M. C. et al. Targeted ablation of glucose-dependent insulinotropic polypeptide-producing cells in transgenic mice reduces obesity and insulin resistance induced by a high fat diet. *J. Biol. Chem.* **283**, 18365–18376 (2008).
38. Killion, E. A. et al. Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models. *Sci. Transl. Med.* **10**, eaat3392 (2018).
39. Newsome, P. N. et al. A placebo-controlled trial of subcutaneous semaglutide in nonalcoholic steatohepatitis. *N. Engl. J. Med.* **384**, 1113–1124 (2021).
40. Landgraf, D. et al. Oxyntomodulin regulates resetting of the liver circadian clock by food. *eLife* **4**, e06253 (2015).
41. McLean, B. A., Wong, C. K., Kaur, K. D., Seeley, R. J. & Drucker, D. J. Differential importance of endothelial and hematopoietic cell GLP-1Rs for cardiometabolic versus hepatic actions of semaglutide. *JCI Insight* **6**, e153732 (2021).
42. Asmar, M. et al. The gluco- and liporegulatory and vasodilatory effects of glucose-dependent insulinotropic polypeptide (GIP) are abolished by an antagonist of the human GIP receptor. *Diabetes* **66**, 2363–2371 (2017).
43. Saari, T. et al. Obesity-associated blunted subcutaneous adipose tissue blood flow after meal improves after bariatric surgery. *J. Clin. Endocrinol. Metab.* **107**, 1930–1938 (2022).
44. Thondam, S. K. et al. Glucose-dependent insulinotropic polypeptide promotes lipid deposition in subcutaneous adipocytes in obese type 2 diabetes patients: a maladaptive response. *Am. J. Physiol. Endocrinol. Metab.* **312**, E224–E233 (2017).
45. Kim, S. J., Nian, C. & McIntosh, C. H. GIP increases human adipocyte LPL expression through CREB and TORC2-mediated trans-activation of the LPL gene. *J. Lipid Res.* **51**, 3145–3157 (2010).

46. Getty-Kaushik, L., Song, D. H., Boylan, M. O., Corkey, B. E. & Wolfe, M. M. Glucose-dependent insulinotropic polypeptide modulates adipocyte lipolysis and reesterification. *Obesity* **14**, 1124–1131 (2006).
47. Hansotia, T. et al. Extrapancreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure. *J. Clin. Invest.* **117**, 143–152 (2007).
48. Svendsen, B. et al. Pharmacological antagonism of the incretin system protects against diet-induced obesity. *Mol. Metab.* **32**, 44–55 (2020).
49. Killion, E. A. et al. Chronic glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism desensitizes adipocyte GIPR activity mimicking functional GIPR antagonism. *Nat. Commun.* **11**, 4981 (2020).
50. Joo, E. et al. Inhibition of gastric inhibitory polypeptide receptor signaling in adipose tissue reduces insulin resistance and hepatic steatosis in high-fat diet-fed mice. *Diabetes* **66**, 868–879 (2017).
51. Campbell, J. E. et al. GIPR is predominantly localized to nonadipocyte cell types within white adipose tissue. *Diabetes* **71**, 1115–1127 (2022).
52. Beaudry, J. L. et al. Physiological roles of the GIP receptor in murine brown adipose tissue. *Mol. Metab.* **28**, 14–25 (2019).
53. Samms, R. J. et al. GIPR agonism mediates weight-independent insulin sensitization by tirzepatide in obese mice. *J. Clin. Invest.* **131**, e146353 (2021).
54. Samms, R. J. et al. Tirzepatide induces a thermogenic-like amino acid signature in brown adipose tissue. *Mol. Metab.* **64**, 101550 (2022).
55. Beiroa, D. et al. GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* **63**, 3346–3358 (2014).
56. van Can, J. et al. Effects of the once-daily GLP-1 analog liraglutide on gastric emptying, glycemic parameters, appetite and energy metabolism in obese, non-diabetic adults. *Int. J. Obes.* **38**, 784–793 (2014).
57. Bollag, R. J. et al. Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology* **141**, 1228–1235 (2000).
58. Zhong, Q. et al. Effects of glucose-dependent insulinotropic peptide on osteoclast function. *Am. J. Physiol. Endocrinol. Metab.* **292**, E543–E548 (2007).
59. Christensen, M. B. et al. Glucose-dependent insulinotropic polypeptide (GIP) inhibits bone resorption independently of insulin and glycemia. *J. Clin. Endocrinol. Metab.* **103**, 288–294 (2018).
60. Torekov, S. S. et al. A functional amino acid substitution in the glucose-dependent insulinotropic polypeptide receptor (GIPR) gene is associated with lower bone mineral density and increased fracture risk. *J. Clin. Endocrinol. Metab.* **99**, E729–E733 (2014).
61. Bjerre Knudsen, L. et al. Glucagon-like peptide-1 receptor agonists activate rodent thyroid C-cells causing calcitonin release and C-cell proliferation. *Endocrinology* **151**, 1473–1486 (2010).
62. Yamada, C. et al. The murine glucagon-like peptide-1 receptor is essential for control of bone resorption. *Endocrinology* **149**, 574–579 (2008).
63. Patorno, E. et al. Comparative effectiveness and safety of sodium-glucose cotransporter 2 inhibitors versus glucagon-like peptide 1 receptor agonists in older adults. *Diabetes Care* **44**, 826–835 (2021).
64. Mantelmacher, F. D. et al. Glucose-dependent insulinotropic polypeptide receptor deficiency leads to impaired bone marrow hematopoiesis. *J. Immunol.* **198**, 3089–3098 (2017).
65. Pujadas, G. et al. The gut hormone receptor GIPR links energy availability to the control of hematopoiesis. *Mol. Metab.* **39**, 101008 (2020).
66. Mantelmacher, F. D. et al. GIP regulates inflammation and body weight by restraining myeloid-cell-derived S100A8/A9. *Nat. Metab.* **1**, 58–69 (2019).
67. Varol, C. et al. Long-acting glucose-dependent insulinotropic polypeptide ameliorates obesity-induced adipose tissue inflammation. *J. Immunol.* **193**, 4002–4009 (2014).
68. Kim, S. J. et al. GIP-overexpressing mice demonstrate reduced diet-induced obesity and steatosis, and improved glucose homeostasis. *PLoS ONE* **7**, e40156 (2012).
69. Chen, S., Okahara, F., Osaki, N. & Shimotoyodome, A. Increased GIP signaling induces adipose inflammation via a HIF-1alpha-dependent pathway and impairs insulin sensitivity in mice. *Am. J. Physiol. Endocrinol. Metab.* **308**, E414–E425 (2015).
70. Gogebakan, O. et al. GIP increases adipose tissue expression and blood levels of MCP-1 in humans and links high energy diets to inflammation: a randomised trial. *Diabetologia* **58**, 1759–1768 (2015).
71. Yusta, B. et al. GLP-1 receptor (GLP-1R) agonists modulate enteric immune responses through the intestinal intraepithelial lymphocyte (IEL) GLP-1R. *Diabetes* **64**, 2537–2549 (2015).
72. Kahles, F. et al. GLP-1 secretion is increased by inflammatory stimuli in an IL-6-dependent manner, leading to hyperinsulinemia and blood glucose lowering. *Diabetes* **63**, 3221–3229 (2014).
73. Ellingsgaard, H. et al. Interleukin-6 enhances insulin secretion by increasing glucagon-like peptide-1 secretion from L cells and alpha cells. *Nat. Med.* **17**, 1481–1489 (2011).
74. Nguyen, A. T. et al. Lipopolysaccharides-mediated increase in glucose-stimulated insulin secretion: involvement of the GLP-1 pathway. *Diabetes* **63**, 471–482 (2014).
75. Lebrun, L. J. et al. Enteroendocrine L cells sense LPS after gut barrier injury to enhance GLP-1 secretion. *Cell Rep.* **21**, 1160–1168 (2017).
76. Kahles, F. et al. Glucagon-like peptide 1 levels predict cardiovascular risk in patients with acute myocardial infarction. *Eur. Heart J.* **41**, 882–889 (2020).
77. Brakenridge, S. C. et al. Persistently elevated glucagon-like peptide-1 levels among critically ill surgical patients after sepsis and development of chronic critical illness and dismal long-term outcomes. *J. Am. Coll. Surg.* **229**, 58–67.e1 (2019).
78. Lebherz, C. et al. GLP-1 levels predict mortality in patients with critical illness as well as end-stage renal disease. *Am. J. Med.* **130**, 833–841.e3 (2017).
79. Noyan-Ashraf, M. H. et al. A glucagon-like peptide-1 analogue reverses the molecular pathology and cardiac dysfunction of a mouse model of obesity. *Circulation* **127**, 74–85 (2013).
80. Wong, C. K. et al. Divergent roles for the gut intraepithelial lymphocyte GLP-1R in control of metabolism, microbiota, and T cell-induced inflammation. *Cell Metab.* **34**, 1514–1531. e7 (2022).
81. Lee, Y. S. et al. Glucagon-like peptide-1 inhibits adipose tissue macrophage infiltration and inflammation in an obese mouse model of diabetes. *Diabetologia* **55**, 2456–2468 (2012).
82. Chaudhuri, A. et al. Exenatide exerts a potent antiinflammatory effect. *J. Clin. Endocrinol. Metab.* **97**, 198–207 (2012).
83. Rodbard, H. W. et al. Oral semaglutide versus empagliflozin in patients with type 2 diabetes uncontrolled on metformin: the PIONEER 2 trial. *Diabetes Care* **42**, 2272–2281 (2019).
84. Hadjiyanni, I., Siminovitch, K. A., Danska, J. S. & Drucker, D. J. Glucagon-like peptide-1 receptor signalling selectively regulates murine lymphocyte proliferation and maintenance of peripheral regulatory T cells. *Diabetologia* **53**, 730–740 (2010).
85. He, S. et al. Gut intraepithelial T cells calibrate metabolism and accelerate cardiovascular disease. *Nature* **566**, 115–119 (2019).
86. Yoon, H. S. et al. *Akkermansia muciniphila* secretes a glucagon-like peptide-1-inducing protein that improves glucose homeostasis and ameliorates metabolic disease in mice. *Nat. Microbiol.* **6**, 563–573 (2021).
87. Chaudhari, S. N. et al. A microbial metabolite remodels the gut-liver axis following bariatric surgery. *Cell Host Microbe* **29**, 408–424.e7 (2021).
88. Grasset, E. et al. A specific gut microbiota dysbiosis of type 2 diabetic mice induces GLP-1 resistance through an enteric NO-dependent and gut-brain axis mechanism. *Cell Metab.* **25**, 1075–1090.e5 (2017).
89. Martchenko, S. E. et al. Circadian GLP-1 secretion in mice is dependent on the intestinal microbiome for maintenance of diurnal metabolic homeostasis. *Diabetes* **69**, 2589–2602 (2020).
90. Heiss, C. N. et al. The gut microbiota regulates hypothalamic inflammation and leptin sensitivity in Western diet-fed mice via a GLP-1R-dependent mechanism. *Cell Rep.* **35**, 109163 (2021).
91. Smits, M. M. et al. Liraglutide and sitagliptin have no effect on intestinal microbiota composition: a 12-week randomized placebo-controlled trial in adults with type 2 diabetes. *Diabetes Metab.* **47**, 101223 (2021).
92. Somm, E. et al. The GLP-1R agonist liraglutide limits hepatic lipotoxicity and inflammatory response in mice fed a methionine-choline deficient diet. *Transl. Res.* **227**, 75–88 (2021).
93. Trevaskis, J. L. et al. Glucagon-like peptide-1 receptor agonism improves metabolic, biochemical, and histopathological indices of nonalcoholic steatohepatitis in mice. *Am. J. Physiol. Gastrointest. Liver Physiol.* **302**, G762–G772 (2012).
94. Armstrong, M. J. et al. Liraglutide safety and efficacy in patients with non-alcoholic steatohepatitis (LEAN): a multicentre, double-blind, randomised, placebo-controlled phase 2 study. *Lancet* **387**, 679–690 (2016).
95. Meier, J. J. et al. Secretion, degradation, and elimination of glucagon-like peptide 1 and gastric inhibitory polypeptide in patients with chronic renal insufficiency and healthy control subjects. *Diabetes* **53**, 654–662 (2004).
96. Idorn, T. et al. Elimination and degradation of glucagon-like peptide-1 and glucose-dependent insulinotropic polypeptide in patients with end-stage renal disease. *J. Clin. Endocrinol. Metab.* **99**, 2457–2466 (2014).
97. Pyke, C. et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* **155**, 1280–1290 (2014).
98. Jensen, E. P. et al. Activation of GLP-1 receptors on vascular smooth muscle cells reduces the autoregulatory response in afferent arterioles and increases renal blood flow. *Am. J. Physiol. Ren. Physiol.* **308**, F867–F877 (2015).
99. Rieg, T. et al. Natriuretic effect by exendin-4, but not the DPP-4 inhibitor alogliptin, is mediated via the GLP-1 receptor and preserved in obese type 2 diabetic mice. *Am. J. Physiol. Ren. Physiol.* **303**, F963–F971 (2012).
100. Gutzwiller, J. P. et al. Glucagon-like peptide 1 induces natriuresis in healthy subjects and in insulin-resistant obese men. *J. Clin. Endocrinol. Metab.* **89**, 3055–3061 (2004).
101. Lovshin, J. A. et al. Liraglutide promotes natriuresis but does not increase circulating levels of atrial natriuretic peptide in hypertensive subjects with type 2 diabetes. *Diabetes Care* **38**, 132–139 (2015).
102. Fujita, H. et al. The protective roles of GLP-1R signaling in diabetic nephropathy: possible mechanism and therapeutic potential. *Kidney Int.* **85**, 579–589 (2014).
103. Ougaard, M. E. et al. Liraglutide improves the kidney function in a murine model of chronic kidney disease. *Nephron* **144**, 595–606 (2020).
104. Moschovaki Filippidou, F. et al. Glucagon-like peptide-1 receptor agonism improves nephrotoxic serum nephritis by inhibiting T-cell proliferation. *Am. J. Pathol.* **190**, 400–411 (2020).
105. Leehey, D. J., Rahman, M. A., Borys, E., Picken, M. M. & Clise, C. E. Acute kidney injury associated with semaglutide. *Kidney Med.* **3**, 282–285 (2021).
106. Chen, J. J. et al. Association of glucagon-like peptide-1 receptor agonist vs dipeptidyl peptidase-4 inhibitor use with mortality among patients with type 2 diabetes and advanced chronic kidney disease. *JAMA Netw. Open* **5**, e221169 (2022).

107. Kristensen, S. L. et al. Cardiovascular, mortality, and kidney outcomes with GLP-1 receptor agonists in patients with type 2 diabetes: a systematic review and meta-analysis of cardiovascular outcome trials. *Lancet Diabetes Endocrinol.* **7**, 776–785 (2019).
108. Gerstein, H. C. et al. Dulaglutide and renal outcomes in type 2 diabetes: an exploratory analysis of the REWIND randomised, placebo-controlled trial. *Lancet* **394**, 131–138 (2019).
109. Shaman, A. M. et al. Effect of the glucagon-like peptide-1 receptor agonists semaglutide and liraglutide on kidney outcomes in patients with type 2 diabetes: pooled analysis of SUSTAIN 6 and LEADER. *Circulation* **145**, 575–585 (2022).
110. Pasternak, B. et al. Use of glucagon-like peptide 1 receptor agonists and risk of serious renal events: Scandinavian cohort study. *Diabetes Care* **43**, 1326–1335 (2020).
111. Baggio, L. L. et al. GLP-1 receptor expression within the human heart. *Endocrinology* **159**, 1570–1584 (2018).
112. Jujic, A. et al. Glucose-dependent insulinotropic peptide in the high-normal range is associated with increased carotid intima–media thickness. *Diabetes Care* **44**, 224–230 (2021).
113. Bowker, N. et al. Genetically predicted glucose-dependent insulinotropic polypeptide (GIP) levels and cardiovascular disease risk are driven by distinct causal variants in the GIPR region. *Diabetes* **70**, 2706–2719 (2021).
114. Nogi, Y. et al. Glucose-dependent insulinotropic polypeptide prevents the progression of macrophage-driven atherosclerosis in diabetic apolipoprotein E-null mice. *PLoS ONE* **7**, e35683 (2012).
115. Pujadas, G. et al. Genetic disruption of the Gipr in Apoe(−/−) mice promotes atherosclerosis. *Mol. Metab.* **65**, 101586 (2022).
116. Karhunen, V. et al. Leveraging human genetic data to investigate the cardiometabolic effects of glucose-dependent insulinotropic polypeptide signalling. *Diabetologia* **64**, 2773–2778 (2021).
117. Terasaki, M. et al. Glucose-dependent insulinotropic polypeptide suppresses foam cell formation of macrophages through inhibition of the cyclin-dependent kinase 5–CD36 pathway. *Biomedicines* **9**, 832 (2021).
118. Kahles, F. et al. The incretin hormone GIP is upregulated in patients with atherosclerosis and stabilizes plaques in ApoE(−/−) mice by blocking monocyte/macrophage activation. *Mol. Metab.* **14**, 150–157 (2018).
119. Mori, Y. et al. Glucose-dependent insulinotropic polypeptide suppresses peripheral arterial remodeling in male mice. *Endocrinology* **159**, 2717–2732 (2018).
120. Hiromura, M. et al. Suppressive effects of glucose-dependent insulinotropic polypeptide on cardiac hypertrophy and fibrosis in angiotensin II-infused mouse models. *Circ. J.* **80**, 1988–1997 (2016).
121. Drucker, D. J. The cardiovascular biology of glucagon-like peptide-1. *Cell Metab.* **24**, 15–30 (2016).
122. Cahill, K. N. et al. Glucagon-like peptide-1 receptor regulates thromboxane-induced human platelet activation. *JACC Basic Transl. Sci.* **7**, 713–715 (2022).
123. Varin, E. M. et al. Distinct neural sites of GLP-1R expression mediate physiological versus pharmacological control of incretin action. *Cell Rep.* **27**, 3371–3384.e3 (2019).
124. Noyan-Ashraf, M. H. et al. GLP-1R agonist liraglutide activates cytoprotective pathways and improves outcomes after experimental myocardial infarction in mice. *Diabetes* **58**, 975–983 (2009).
125. Mclean, B. A., Wong, C. K., Kabir, M. G. & Drucker, D. J. Glucagon-like peptide-1 receptor Tie2+ cells are essential for the cardioprotective actions of liraglutide in mice with experimental myocardial infarction. *Mol. Metab.* https://doi.org/10.1016/j.molmet.2022.101641 (2022).
126. Ussher, J. R. et al. Inactivation of the cardiomyocyte glucagon-like peptide-1 receptor (GLP-1R) unmasks cardiomyocyte-independent GLP-1R-mediated cardioprotection. *Mol. Metab.* **3**, 507–517 (2014).
127. Nagashima, M. et al. Native incretins prevent the development of atherosclerotic lesions in apolipoprotein E knockout mice. *Diabetologia* **54**, 2649–2659 (2011).
128. Rakipovski, G. et al. The GLP-1 analogs liraglutide and semaglutide reduce atherosclerosis in ApoE(−/−) and LDLr(−/−) mice by a mechanism that includes inflammatory pathways. *JACC Basic Transl. Sci.* **3**, 844–857 (2018).
129. Helmstadter, J. et al. Endothelial GLP-1 (glucagon-like peptide-1) receptor mediates cardiovascular protection by liraglutide in mice with experimental arterial hypertension. *Arterioscler. Thromb. Vasc. Biol.* **40**, 145–158 (2020).
130. Cherney, D. Z. I., Udell, J. A. & Drucker, D. J. Cardiorenal mechanisms of action of glucagon-like-peptide-1 receptor agonists and sodium-glucose cotransporter 2 inhibitors. *Med (N Y)* **2**, 1203–1230 (2021).
131. Gerstein, H. C. et al. Cardiovascular and renal outcomes with efpeglenatide in type 2 diabetes. *N. Engl. J. Med.* **385**, 896–907 (2021).
132. Dave, C. V. et al. Risk of cardiovascular outcomes in patients with type 2 diabetes after addition of SGLT2 inhibitors versus sulfonylureas to baseline GLP-1RA therapy. *Circulation* **143**, 770–779 (2021).
133. Ryan, D. H. et al. Semaglutide effects on cardiovascular outcomes in people with overweight or obesity (SELECT) rationale and design. *Am. Heart J.* **229**, 61–69 (2020).
134. Ripa, R. S. et al. Effect of liraglutide on arterial inflammation assessed as [(18)F]FDG uptake in patients with type 2 diabetes: a randomized, double-blind, placebo-controlled trial. *Circ. Cardiovasc. Imaging* **14**, e012174 (2021).
135. Jensen, J. K. et al. Effect of 26 weeks of liraglutide treatment on coronary artery inflammation in type 2 diabetes quantified by [(64)Cu]Cu-DOTATATE PET/CT: results from the LIRAFLAME trial. *Front. Endocrinol.* **12**, 790405 (2021).
136. Koska, J., Migrino, R. Q., Chan, K. C., Cooper-Cox, K. & Reaven, P. D. The effect of exenatide once weekly on carotid atherosclerosis in individuals with type 2 diabetes: an 18-month randomized placebo-controlled study. *Diabetes Care* **44**, 1385–1392 (2021).
137. Zobel, E. H. et al. Effect of liraglutide on expression of inflammatory genes in type 2 diabetes. *Sci. Rep.* **11**, 18522 (2021).
138. Nyberg, J. et al. Glucose-dependent insulinotropic polypeptide is expressed in adult hippocampus and induces progenitor cell proliferation. *J. Neurosci.* **25**, 1816–1825 (2005).
139. Nyberg, J., Jacobsson, C., Anderson, M. F. & Eriksson, P. S. Immunohistochemical distribution of glucose-dependent insulinotropic polypeptide in the adult rat brain. *J. Neurosci. Res.* **85**, 2099–2119 (2007).
140. Adriaenssens, A. E. et al. Glucose-dependent insulinotropic polypeptide receptor-expressing cells in the hypothalamus regulate food intake. *Cell Metab.* **30**, 987–996.e6 (2019).
141. Smith, C. et al. A comparative transcriptomic analysis of glucagon-like peptide-1 receptor- and glucose-dependent insulinotropic polypeptide-expressing cells in the hypothalamus. *Appetite* **174**, 106022 (2022).
142. Ludwig, M. Q. et al. A genetic map of the mouse dorsal vagal complex and its role in obesity. *Nat. Metab.* **3**, 530–545 (2021).
143. Bates, H. E. et al. Gipr is essential for adrenocortical steroidogenesis; however, corticosterone deficiency does not mediate the favorable metabolic phenotype of Gipr(−/−) mice. *Diabetes* **61**, 40–48 (2012).
144. Kaneko, K. et al. Gut-derived GIP activates central Rap1 to impair neural leptin sensitivity during overnutrition. *J. Clin. Invest.* **130**, 3786–3791 (2019).
145. Zhang, Q. et al. The glucose-dependent insulinotropic polypeptide (GIP) regulates body weight and food intake via CNS-GIPR signaling. *Cell Metab.* **33**, 833–844.e5 (2021).
146. Drucker, D. J. GLP-1 physiology informs the pharmacotherapy of obesity. *Mol. Metab.* **57**, 101351 (2022).
147. Zhang, T., Perkins, M. H., Chang, H., Han, W. & de Araujo, I. E. An inter-organ neural circuit for appetite suppression. *Cell* **185**, 2478–2494.e28 (2022).
148. Nauck, M. A., Kemmeries, G., Holst, J. J. & Meier, J. J. Rapid tachyphylaxis of the glucagon-like peptide 1-induced deceleration of gastric emptying in humans. *Diabetes* **60**, 1561–1565 (2011).
149. Plamboeck, A. et al. The effect of exogenous GLP-1 on food intake is lost in male truncally vagotomized subjects with pyloroplasty. *Am. J. Physiol. Gastrointest. Liver Physiol.* **304**, G1117–G1127 (2013).
150. Gabery, S. et al. Semaglutide lowers body weight in rodents via distributed neural pathways. *JCI Insight* **5**, e133429 (2020).
151. Burmeister, M. A. et al. The hypothalamic glucagon-like peptide 1 receptor is sufficient but not necessary for the regulation of energy balance and glucose homeostasis in mice. *Diabetes* **66**, 372–384 (2017).
152. Fortin, S. M. et al. GABA neurons in the nucleus tractus solitarius express GLP-1 receptors and mediate anorectic effects of liraglutide in rats. *Sci. Transl. Med.* **12**, eaay8071 (2020).
153. Duffy, A. M. & Holscher, C. The incretin analogue D-Ala(2)GIP reduces plaque load, astrogliosis and oxidative stress in an APP/PS1 mouse model of Alzheimer's disease. *Neuroscience* **228**, 294–300 (2013).
154. Faivre, E., Hamilton, A. & Holscher, C. Effects of acute and chronic administration of GIP analogues on cognition, synaptic plasticity and neurogenesis in mice. *Eur. J. Pharmacol.* **674**, 294–306 (2012).
155. Porter, D. W., Irwin, N., Flatt, P. R., Holscher, C. & Gault, V. A. Prolonged GIP receptor activation improves cognitive function, hippocampal synaptic plasticity and glucose homeostasis in high-fat fed mice. *Eur. J. Pharmacol.* **650**, 688–693 (2011).
156. Li, Y., Liu, W., Li, L. & Holscher, C. D-Ala2-GIP-glu-PAL is neuroprotective in a chronic Parkinson's disease mouse model and increases BNDF expression while reducing neuroinflammation and lipid peroxidation. *Eur. J. Pharmacol.* **797**, 162–172 (2017).
157. Verma, M. K., Goel, R., Nandakumar, K. & Nemmani, K. V. S. Bilateral quinolinic acid-induced lipid peroxidation, decreased striatal monoamine levels and neurobehavioral deficits are ameliorated by GIP receptor agonist D-Ala(2)GIP in rat model of Huntington's disease. *Eur. J. Pharmacol.* **828**, 31–41 (2018).
158. Yuan, L. et al. DAla2-GIP-GLU-PAL protects against cognitive deficits and pathology in APP/PS1 mice by inhibiting neuroinflammation and upregulating cAMP/PKA/CREB signaling pathways. *J. Alzheimers Dis.* **80**, 695–713 (2021).
159. Yang, X. et al. Neuroprotective mechanisms of glucagon-like peptide-1-based therapies in ischemic stroke: an update based on preclinical research. *Front. Neurol.* **13**, 844697 (2022).
160. Aviles-Olmos, I. et al. Exenatide and the treatment of patients with Parkinson's disease. *J. Clin. Invest.* **123**, 2730–2736 (2013).
161. Athauda, D. et al. Exenatide once weekly versus placebo in Parkinson's disease: a randomised, double-blind, placebo-controlled trial. *Lancet* **390**, 1664–1675 (2017).
162. Vijiaratnam, N. et al. Exenatide once weekly over 2 years as a potential disease-modifying treatment for Parkinson's disease: protocol for a multicentre, randomised, double blind, parallel group, placebo controlled, phase 3 trial: the 'Exenatide-PD3' study. *BMJ Open* **11**, e047993 (2021).
163. Watson, K. T. et al. Neural correlates of liraglutide effects in persons at risk for Alzheimer's disease. *Behav. Brain Res.* **356**, 271–278 (2019).
164. Cukierman-Yaffe, T. et al. Effect of dulaglutide on cognitive impairment in type 2 diabetes: an exploratory analysis of the REWIND trial. *Lancet Neurol.* **19**, 582–590 (2020).
165. Norgaard, C. H. et al. Treatment with glucagon-like peptide-1 receptor agonists and incidence of dementia: data from pooled double-blind randomized controlled trials and nationwide disease and prescription registers. *Alzheimers Dement.* **8**, e12268 (2022).

166. Gasbjerg, L. S. et al. Separate and combined glucometabolic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals. *Diabetes* **68**, 906–917 (2019).
167. Frias, J. P. et al. Tirzepatide versus semaglutide once weekly in patients with type 2 diabetes. *N. Engl. J. Med.* **385**, 503–515 (2021).
168. Jastreboff, A. M. et al. Tirzepatide once weekly for the treatment of obesity. *N. Engl. J. Med.* **387**, 205–216 (2022).
169. Gastaldelli, A. et al. Effect of tirzepatide versus insulin degludec on liver fat content and abdominal adipose tissue in people with type 2 diabetes (SURPASS-3 MRI): a substudy of the randomised, open-label, parallel-group, phase 3 SURPASS-3 trial. *Lancet Diabetes Endocrinol.* **10**, 393–406 (2022).
170. Finan, B. et al. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci. Transl. Med.* **5**, 209ra151 (2013).
171. Mroz, P. A. et al. Optimized GIP analogs promote body weight lowering in mice through GIPR agonism not antagonism. *Mol. Metab.* **20**, 51–62 (2019).
172. Bergmann, N. C. et al. Effects of combined GIP and GLP-1 infusion on energy intake, appetite and energy expenditure in overweight/obese individuals: a randomised, crossover study. *Diabetologia* **62**, 665–675 (2019).
173. Yuan, Z. et al. A novel GLP-1/GIP dual agonist is more effective than liraglutide in reducing inflammation and enhancing GDNF release in the MPTP mouse model of Parkinson's disease. *Eur. J. Pharmacol.* **812**, 82–90 (2017).
174. Li, T. et al. A GLP-1/GIP/Gcg receptor triagonist improves memory behavior, as well as synaptic transmission, neuronal excitability and Ca(2+) homeostasis in 3xTg-AD mice. *Neuropharmacology* **170**, 108042 (2020).
175. Borner, T. et al. GIP receptor agonism attenuates GLP-1 receptor agonist induced nausea and emesis in preclinical models. *Diabetes* **70**, 2545–2553 (2021).
176. Zhang, C., Vincelette, L. K., Reimann, F. & Liberles, S. D. A brainstem circuit for nausea suppression. *Cell Rep.* **39**, 110953 (2022).
177. Wilson, J. M. et al. The dual glucose-dependent insulinotropic polypeptide and glucagon-like peptide-1 receptor agonist tirzepatide improves cardiovascular risk biomarkers in patients with type 2 diabetes: a post hoc analysis. *Diabetes Obes. Metab.* **24**, 148–153 (2022).
178. Sattar, N. et al. Tirzepatide cardiovascular event risk assessment: a pre-specified meta-analysis. *Nat. Med.* **28**, 591–598 (2022).
179. Miyawaki, K. et al. Glucose intolerance caused by a defect in the entero-insular axis: a study in gastric inhibitory polypeptide receptor knockout mice. *Proc. Natl Acad. Sci. USA* **96**, 14843–14847 (1999).
180. Lu, S. C. et al. GIPR antagonist antibodies conjugated to GLP-1 peptide are bispecific molecules that decrease weight in obese mice and monkeys. *Cell Rep. Med.* **2**, 100263 (2021).
181. N'Diaye, N., Tremblay, J., Hamet, P., De Herder, W. W. & Lacroix, A. Adrenocortical overexpression of gastric inhibitory polypeptide receptor underlies food-dependent Cushing's syndrome. *J. Clin. Endocrinol. Metab.* **83**, 2781–2785 (1998).
182. Regazzo, D. et al. The GIP/GIPR axis is functionally linked to GH-secretion increase in a significant proportion of gsp(−) somatotropinomas. *Eur. J. Endocrinol.* **176**, 543–553 (2017).
183. Harada, N. et al. A novel GIP receptor splice variant influences GIP sensitivity of pancreatic beta-cells in obese mice. *Am. J. Physiol. Endocrinol. Metab.* **294**, E61–E68 (2008).
184. Ahlqvist, E. et al. Link between GIP and osteopontin in adipose tissue and insulin resistance. *Diabetes* **62**, 2088–2094 (2013).
185. DeBoever, C. et al. Medical relevance of common protein-altering variants in GPCR genes across 337,205 individuals in the UK Biobank. Preprint at *bioRxiv* https://doi.org/10.1101/2019.12.13.876250 (2020).
186. Harris, M. et al. RAMPs regulate signalling bias and internalisation of the GIPR. Preprint at *bioRxiv* https://doi.org/10.1101/2021.04.08.436756 (2021).
187. Lagou, V. et al. Random glucose GWAS in 493,036 individuals provides insights into diabetes pathophysiology, complications and treatment stratification. Preprint at *medRxiv* https://doi.org/10.1101/2021.04.17.21255471 (2021).
188. Dawed, A. Y. et al. Pharmacogenomics of GLP-1 receptor agonists: a genome wide analysis of observational data and large randomized controlled trials. Preprint at *medRxiv* https://doi.org/10.1101/2022.05.27.22271124 (2022).

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