Dissecting the Physiology and Pathophysiology of Glucagon-Like Peptide-1

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An aging world population exposed to a sedentary lifestyle is currently plagued by chronic metabolic diseases, such as type-2 diabetes, that are spreading worldwide at an unprecedented rate. One of the most promising pharmacological approaches for the management of type 2 diabetes takes advantage of the peptide hormone glucagon-like peptide-1 (GLP-1) under the form of protease resistant mimetics, and DPP-IV inhibitors. Despite the improved quality of life, long-term treatments with these new classes of drugs are riddled with serious and life-threatening side-effects, with no overall cure of the disease. New evidence is shedding more light over the complex physiology of GLP-1 in health and metabolic diseases. Herein, we discuss the most recent advancements in the biology of gut receptors known to induce the secretion of GLP-1, to bridge the multiple gaps into our understanding of its physiology and pathology.

Keywords: glucagon-like peptide-1, metabolic disease, type 2 diabetes, enteroendocrine cell system, GPCR, L-cells, microbiome, α-cells

INTRODUCTION

The gastrointestinal (GI) tract is a complex organ that monitors the body’s energetical state and provides it with water and macrominerals and micronutrients extracted from the ingested food. Along its length, the enteroendocrine cells (EECs) constitute a complex endocrine organ that communicates with the central nervous system (CNS) and the enteric nervous system (ENS) to orchestrate the homeostatic balance of the body in response to the GI luminal content.

This enteroendocrine system has traditionally been divided into 12 different cell types, based entirely on their hormonal content and cellular morphology. This endocrine organ is not organized in a glandular structure; on the contrary, it is dispersed heterogeneously, mainly as single cells, along the epithelium of the GI tract, from the stomach to the rectum with a defined cephalocaudal, crypt-to-villus in the small intestine and crypt-to-surface distribution in the colon (1, 2).

Despite representing just 1% of the adult gut epithelium, in the last decade it has become clear that the EECs constitute the largest endocrine organ in mammalia (3). Recent analysis of the expression of specific hormones at the cellular level, demonstrated that the EECs subdivision introduced above is outdated. Each enteroendocrine cell co-secretes multiple hormones with spatio-temporal, crypt-to-villus, and rostro-caudal variability, leading to the formation of overlapped gradients of individual hormones along the GI tract; the concept of well-defined subclasses of cells committed to express a specific subset of hormones independent of their location is currently untenable, thus detailed description of the topographical location of the cells needs to be implemented for future clarity (4).
Collectively, the EECs are responsible for the production of more than 30 different hormones that help to orchestrate the fate of the intermediary metabolism; acting upon different organs such as the pancreatic islets, the hypothalamus or the stomach, for the release of insulin, to regulate food intake or gastric emptying respectively (5–8).

Surprisingly, this heterogeneous and highly plastic population of cells is known to differentiate from a single staminal progenitor that gives also rise to enterocytes, goblet and paneth cells (1, 9).

It has been known for more than a century that the gut is capable to stimulate the endocrine portion of the pancreas and even improve the hyperglycaemic state of diabetic patients (10, 11). In 1932, the Belgian investigator LaBarre referred to these “factors” extracted from the intestinal mucosa as “incrétine,” deriving it from: INtestinal sCRETiOn of insulin (12). In the 60s, different authors demonstrated that oral glucose was capable to induce a 2-fold increase in insulin compared to an in-vein isoglycaemic administration (13).

In the last three decades, the incretin-effect has been attributed primarily to two peptide hormones, the gastric-insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), excreted primarily by duodenal (K) and ileo-colonic (L) enteroendocrine cells respectively (14). Indeed, type 2 diabetes (T2D) is a metabolic disease reported to involve an impaired intestinal release of GLP-1 and its co-secreted peptides oxyntomodulin and glicentin (15–17), together with an insulinogetic resistance to GIP in the pancreas (18) which lead to a deficient incretin system, purportedly causing the disease (19, 20). Despite being still largely unknown how hyper caloric diets are disrupting the incretin signaling, some authors have shown that even circadian rhythms disruption, and the saturated fat palmitate, are significant stressors capable to hamper GLP-1 secretion (21, 22).

Obesity and Type 2 diabetes are chronic diseases for which the most effective treatment is bariatric surgery. These invasive gut surgical procedures, aimed to reduce absorptive surface area of the proximal GI tract, such as Roux-en-Y gastric by-pass (RYGB) or Sleeve Gastrectomy (SLG), are associated with an improved glycaemic control, weight loss, and often with complete remission from T2DM (23).

Despite this, the complete remittance of a great fraction of RYGB patients represents a fascinating new case-series that points at the importance of the EECs and its modulation of the whole-body metabolism (24). As such, the study of this complex endocrine organ, might help us to create new pharmacological tools to amend the specific molecular axis that drive T2DM and the associated co-morbidities known to affect the cardiovascular (25, 26) and renal system (27, 28).

A panoply of contradictory studies have attempted to establish what is the possible role of GLP-1 or other gut peptides in the rapid, and long-lasting remittance from T2D after bariatric surgery, but no consensus about the identity of the molecular players has yet been reached (29–39).

Since 2005, there are on the market only two classes of drugs that attempt to bolster glucagon-like peptide-1 signaling, GLP-1 receptor agonists and DPP-IV inhibitors, for a supra-physiological GLP-1 activity. Unexpected safety-issues and important side-effects (40) prove that the peripheral hijack of this peptide is not sufficient, and does not replicate the remittance seen in bariatric surgery.

This review summarizes the most recent studies that reframe our understanding of the physiology of GLP-1 in health and disease.

CHEMOSENSATION IN GLP-1-PRODUCING CELLS

Intestinal proglucagon expressing cells were historically named L-cells more than 4 decades ago because of their large 500 nm secretory granules seen under electron microscopy (41). Today, we know that these are nutrient-responsive enteroendocrine cells that secrete a variety of peptide hormones, primarily derived from the proglucagon gene (GCG) (42). Once translated, the 180 amino acid long GCG protein is processed by two proteases, Psck1 and Psck3, to give GLP-1, GLP-2 but also the less studied GCGPsck1 and GCGPsck2, to give GLP-1, GLP-2 but also the less studied GCGPsck1 and GCGPsck2 (43).

Other peptide hormones, such as insulin-like peptide 5 (INSL5) (44, 45), PYY (46), GIP and neurotensin (17, 47) can be co-expressed with the GCG products depending on the topographical localization of the cell; surprisingly, it appears that GLP-1 and PYY can be excreted independently possibly due to the existence of compartmentalized secretory vesicles (48).

There appear to be considerable species-specificity in terms of anatomical localization of GLP-1 production as summarized in Figure 1. Independently of other hormones, in mice the distal colon and rectum show the higher levels of GLP-1 per gram of tissue. Conversely, in rats the distal ileum and in pigs the caecum are the anatomical regions with the highest amounts of GLP-1 (49). In humans, the density of GLP-1 and PYY positive cells increase steadily along the small intestine, decreasing in the colon, and then raising again reaching a maximum density in the rectum with the highest values of around 150 GLP-1-expressing cells per square millimeter. Curiously in type 2 diabetes, an equally distributed gradient of GCG and PC1/3 mRNA appears upregulated, but with normal GLP-1+ cell densities, indicating a possible translational resistance (51).

The L-cells derived cocktail of hormones is believed to play pivotal roles in digestion, for example slowing down the GI motility (PYY) and suppressing the appetite in vivo (GLP-1, oxyntomodulin, PYY), apparently in response to direct sensing of the gut luminal content via G-protein coupled receptors or through neuronal circuits (43, 52).

Current in vitro technologies are not capable to support for long-term ex vivo the growth of isolated GLP-1 producing-cells. The available knowledge about the biology of GLP-1 is primarily drawn upon studies operated with the murine-derived GLUTag or STC-1, and the human-derived NCI-H716 cell lines. It is important to understand that these in vitro models express a different hormonal cocktail and respond to different chemical stimuli than intestinal L-cells in vivo (53, 54). Primary cultures are another useful short-term system; nonetheless GLP-1-producing cells amount to only 1–2% of the whole cultured mucosal population, with considerable intra and inter-assay variability (53).
FIGURE 1 | Intestinal glucagon-like peptide-1 expression across species. Total GLP-1 expression along the rat, mouse, pig and human intestinal tracts (relative lengths not to scale) is displayed with gradients as individually indicated in figure. The rat GI tract shows the highest levels of GLP-1 in the ileum and proximal colon. On the other hand the murine gut, displays the highest GLP-1 levels in the distal colon. The porcine intestine shows highest levels in the caecum and distal colon, and virtually none in the proximal small intestine. In humans, a steady increasing gradient along the small intestine is followed by a decrease in expression in the colon, and a second steeper gradient culminating in the rectum with the highest GLP-1 expression (49–51).

The more physiologically relevant studies make use of in vivo transgenic mice, ex vivo perfused intestines or, more recently, crypt organoids derived from human, mouse or porcine guts (55). In situ immunostaining and FACS studies have demonstrated that the hormonal secretome of GLP-1-secreting-cells is anatomically dependent. In the upper gut where these cells are more sparse and rare, GLP-1 is co-expressed with GIP, a K-cell feature, but also with cholecystokinin (CCK) and Neuropeptide Y (NT). Conversely in the colonic mucosa, GLP-1 co-localizes with PYY, CCK and the orexigenic Insulin-Like peptide 5 (INSL5) (4, 43, 53, 56, 57). Interestingly, colonic L-cells possess twice as much total GLP-1 compared to L-cells from the upper GI tract (53). Furthermore, considering the differential response to glucose, it is clear that the physiology of this population of EECs is distinct, and evolved under a different evolutionary pressure dictated by the exposure to a different luminal content (53, 58).

L-cells are known to modulate the release of their hormonal cargo in response to the activation of a plethora of receptors capable to sense fats, carbohydrates, proteins and many other compounds. Enteroendocrine cells, like other endocrine, muscle and neuronal cells, are electrically excitable. Membrane depolarization, triggered by a ligand-bound receptor, results in a spike of intracellular calcium ($Ca^{2+}$) which leads to the fusion of the endocrine granules with the lateral and the broader basal side, resulting in the discharge of a hormonal cargo in the capillaries of the mucosa.

Surprisingly, the EECs in the colon have been demonstrated to physically connect through a basal process named Neuropod, with afferent nerve cells residing in the lamina propria, defining a neuroepithelial circuit that expands the physiology of these cells (59). In fact, the idea of a direct neuronal regulation has been demonstrated decades ago in rats, where a bilateral vagotomy massively downregulates circulating PYY and GLP-1 levels after a glucose load (60). Furthermore, intracerebral acute, but not chronic administration of GLP-1 in mice, improves pancreatic glucose stimulated insulin secretion (61).

**GPCRs AS MOLECULAR TASTANTS**

G-protein coupled receptors (GPCRs) are evolutionary ancient proteins spanning seven times across the plasma membrane of virtually any known cell type. In metazoans, these proteins evolved into thousands different molecular transducers capable to translate the presence of extracellular molecules into intracellular cascades of messages amplified by different G-proteins, which in turn enforce a myriad of different cellular processes via secondary messengers (62). The transmembrane domain of these chemosensors being exposed to a tighter evolutionary pressure lead to a relative evolutionary stability of the same 3-dimensional structure. On the contrary, the extracellular facing portion is what primarily defines the identity of a myriad of different receptors, capable to sense a panoply
of molecular entities ranging in size from a single atom to hundreds aminoacids long proteins. The intracellular portion of these nano-sensors, has evolved in humans in a complex hub that triggers multiple molecular cascades that results in short-term and long-term modifications of the target cell and even the whole-body metabolism.

Different receptors, expressed by the same cell type or tissue, can trigger the same molecular cascade. With this notion, the study of these molecular transducers has been approached by some authors in recent years from a top-down point of view, whereby sub-type specific, allosteric positive or negative modulators (PAM, NAMs), as well as direct agonists, are utilized as tools for pathway dissection and analysis (63, 64). In the last decade, technological advancements in techniques such as circular dichroism (65), Cryo-electron microscopy (Cryo-EM) (66) and crystallography (67) have expanded our understanding of the physiology of multiple chemosensors expressed by L-cells, which led to the discovery of new molecular tools with possible future clinical applications in diseases such as type 2 diabetes (64, 68–70).

The expression of different GPCRs to restricted anatomical regions, such as the enteroendocrine cell system, is a finely tuned system that evolved in metazoan. Macronutrients, bile acids (BAs), and microbiota-derived compounds activate many of these GPCRs expressed by GLP-1 expressing cells (71). Nonetheless, not all intestinal stimuli signals through these chemosensors; for example glucose induces the release of GLP-1 from human duodenum and ileum via electrogenic transporters (SGLT1) and voltage-gated Calcium and Sodium channels responsible for the membrane depolarization and hormonal release (53, 72).

The main G protein-coupled receptors which activation appears to cause the release of GLP-1 are: GPRC6A (73), GPR40-41-42-43-93-119-120 (43), GPR142, GHS-R1A (74), Tas1R2-Tas2R3(T1R2-T1R3) (75), GBP BAR1 (TGR5), and CasR (6, 76, 77) (Table 1). The functional differences seen between Jejunum-ileal and colonic GLP-1 producing cells, could be explained by a different pool of GPCRs, or possibly by the presence of heteromers displaying a more complex pharmacology than with each individual receptor.

A summary of the recognized main activities of all the major GLP-1-secreting receptors, including the GIPR (93, 94), is shown in Table 1.

Many of these chemosensors are also expressed by other enterendocrine cells, so that the same dietary ligand traveling along the GI tract, leads to the release of multiple hormones.

There are some receptors, such as GPRC6A, with a pleiotropic distribution and still a limited understanding of its physiology. GPRC6A is highly expressed in GLUTag cells, and its activation by L-ornithine has shown to induce GLP-1 secretion (102). Nonetheless, mice deficient for the receptor, show no difference in responsiveness to both L-ornithine and L-arginine (103).

### THE PHYSIOLOGY OF GLP-1

In the last three decades a major tenet seeing GLP1 (7-36)NH2, GLP1 (7-37) and the Gastric Insulinotropic Peptide (GIP) as the major contributors of the physiological incretin effect has reached widespread consensus (104). The remaining Glucose-stimulated insulin secretion (GSIS) appears to be enhanced by nutrients, hormones such as CCK, bile acids and endogenous ethanolamides. Animal models show compensatory mechanisms by which, in absence of a major incretin axis, other minor pathways are promoted in the β-cells to maintain their metabolic activity; namely proteins such as GPR119, or the CCK A receptor itself are upregulated, implying a highly plastic metabolic adaptation (105).

Multiple cell types found in the enteroendocrine cell system, the pancreatic islets or the brain have been shown to express the GCG product, a 180 aminoacids long peptide known as proglucagon (PG) (106, 107), which gets trimmed tissue-dependently into at least 6 different bio-active peptides, namely glicentin, oxyntomodulin, glucagon, miniglucagon, GLP-1 and GLP-2 (108, 109). The post-translational processing of the preproglucagon gene into the individual peptides is controlled by two distinct serine proteases, specifically prohormone convertases named Psck1/3 and Psck2, also known as PC1/3, or just PC1, and PC2 respectively (107, 108, 110). PC1/3 and PC2 are responsible for the metabolism of a plethora of peptide pro-hormones, including insulin and GCG among others (111).

In particular PC1/3 expressing cells, such as intestinal L-cells and pancreatic β-cells, produce GLP-1, GLP-2 oxyntomodulin and glicentin (110, 112), while PC2 action on PG results in the production of glucagon and its active metabolite mini-glucagon (113, 114). Differential expression of PC genes regulates the hormonal output, and indeed it has been proven that both are expressed along the intestine, with PC1/3 positive cells found more distally than PC2 expressing cells (51), likely secreting glucagon (115). Indeed, theRYGB surgery removes the biggest pool of PC2/glucacon expressing cells from the exposure to nutrients, possibly contributing to the surgical success.

Active GLP-1(7-37), in human and mice is largely metabolized by the enzyme peptidyl-glycine α-amidating monoxygenase (PAM) into the equally active GLP-1(7-36)NH2 (49, 116). Both these peptide species are trimmed at their N-term, and inactivated by the ubiquitous protease dipeptidyl-peptidase-IV (DPP-IV), found in the intestinal capillaries, vena porta and liver. Indeed, it has been estimated that just 10-15% of the secreted GLP-1(7-36)NH2 reaches the systemic circulation (117), with some authors reporting meager peripheral meal-induced changes in both healthy and diabetic people (118). Furthermore, the DPP-IV product, GLP-1(9-36)NH2, is trimmed into GLP-1(28-36)NH2 and GLP-1(32-36)NH2 by another ubiquitous protease, known as NEP24.11, CD10 or also Nephrilysin among other names (119, 120).

Indeed, these once thought inactive metabolites of the recognized GLP-1 receptor agonist GLP-1(7-36) NH2 have recently shown to possess multiple beneficial properties. The 9 aminoacids long GLP-1(1-28-36) protects β-cells from glucolipotoxicity (121), diet-induced steatosis of the liver (122), improves hepatic glucose tolerance in diabetic mice (122–124). Similarly, the 5 aminoacids long GLP-1(32-36)NH2 improves glucose disposal, increases energy expenditure and protects β-cells in a diabetic environment in vivo (125–127). Indeed GLP-1(9-36) pharmacodynamics studies in human might be partially explained by the activity of its metabolites (128).
These metabolites have possibly important implications for any future treatment of metabolic pathologies such as type 2 diabetes, where our understanding of the pharmacokinetic and pharmacodynamics in humans is virtually absent (128).

In healthy humans, intact GLP-1(7-36)NH₂ is mainly released by intestinal EECs after the ingestion of food, especially meals rich in fat and proteins (14, 129). Other stimuli, such as physical activity, are also capable to raise its plasmatic levels for up to 90 min after exercise (130).

This hormone generates both short-term and long-term pleiotropic effects. GLP-1 stimulates the β-cells to produce Insulin, blocks pancreatic α-cells’ glucagon release via somatostatin (96), slows down gastric emptying (131), improves peripheral glucose tolerance (132), suppresses appetite in the hypothalamus and amygdala (97), increases β-cell mass, GSIS, and elicits protection from glucolipotoxicity (133) and apoptosis (134). Curiously, it also regulates bone physiology (135), and shows anti-inflammatory properties (136).

On the other hand, the most abundant DPP-IV-processed metabolite GLP-1 (9-36)NH₂, has also been reported to have biological activities, protecting human aortic endothelial cells and cardiomyocytes in vivo in dogs (137) and ex vivo in mice (138) and rats (139), even in the absence of a GLP-1 Receptor (139, 140). Some authors postulate the existence of an unknown GLP-1(9-36)NH₂ receptor (141, 142), because indeed this cleaved peptide is found in peripheral blood at one order of magnitude higher concentrations than “active” GLP-1 (7-36)NH₂ and shows cardioprotection, antioxidant properties (138) and appears capable to also inhibit hepatic neoglucogenesis (141).

GLP-1 (7-36)NH₂ itself is known to have general protective and modulating cardiovascular effects (143), as shown by different commercial GLP-1 mimics with proven cardioprotection type 2 diabetes (144).

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**TABLE 1 | Demonstrated primary effects of the major GLP-1-stimulating receptors.**

| Receptor/GPR | Ligand | Effect | Experimental condition | References |
|--------------|--------|--------|------------------------|------------|
| FFAR1/GPR40  | Palmitate | Insulin †, glucagon †, somatostatin† | Ex-vivo human islets | (78) |
|              | Free fatty acids | GLP-1 †, GIP † | In-vivo mouse | (79) |
|              | Long chain fatty acids | CCK † | Ex-vivo murine duodenal I cells | (80) |
| FFAR2/GPR43  | Inulin | PYY † | In-vivo diabetic mouse | (81) |
|              | Propionate | PYY †, GLP-1 † | Ex-vivo murine colonic Primary cultures, & in-vivo murine and rat | (82) |
| FFAR3/GPR41  | Propionate | PYY †, GLP-1 † | Ex-vivo murine colonic primary cultures | (83) |
| FFAR4/GPR120 | α-Linolenic acid | GLP-1 † | In-vivo mouse | (84) |
|              | Lard oil, corn oil | GIP †, CCK † | In-vivo mouse | (85, 86) |
| GPR119       | Oleoyl-LP, OEA | GLP-1 † | In-vitro murine GLUTag, ex-vivo human colon | (87, 88) |
|              | AR231453, AR435707, AR440006, OEA, 2-OG | PYY †, GLP-1 †, Gl motility ↓ | Ex-vivo murine gut, in-vivo healthy and diabetic mouse, ex-vivo human colon | (89, 90) |
|              | Hypogl. + AR231453 | Insulin †, Glmotility ↓ | In-vitro murine MIN6 | (88) |
|              | Hypogl. * Compounds A/B^A | Insulin † | Ex-vivo rat pancreas | (91) |
|              | Hypogl. ** Compounds A/B^A | Glucagon † | Ex-vivo rat pancreas | (91) |
|              | DS-8500a | Insulin †, glucagon †, GLP-1 †, GIP †, PYY ↓ | Type 2 diabetic humans | (92) |
| GIPR         | Hypogl. ** + GIP | Glucagon † | Type 1 diabetic humans | (93) |
|              | Hypogl. ^* + GIP | Insulin †, somatostatin† | Healthy humans | (94) |
|              | GIP | IL-6 † | Ex-vivo human, and murine α-cells | (95) |
| GLP-IR       | GLP-1 | Insulin †, somatostatin†, glucagon ↓ | Ex-vivo healthy murine pancreas | (96) |
|              | GLP-1 | Appetite ↓ | In-vivo intracerebral rat | (97) |
|              | GLP-1 | GLP-1 † | In-vitro murine α-TC 1-6 | (98) |
|              | Exendin-4 | Glucagon ↓ | Ex-vivo healthy rat pancreas | (99) |
|              | Exendin-4 | Glucagon ↑ | Ex-vivo diabetic rat pancreas | (99) |
| TGR5         | Hypogl. ^* + INT-777 ^, or LCA$ | GLP-1 †, insulin ↑ | Ex-vivo healthy human, and murine diabetic islets | (100) |
|              | Taurodeoxycholate | GLP-1 † | Ex-vivo murine primary ileal cultures | (101) |

*Analyses are indicated as up (^) or down (▼) regulated. All in-vivo, or in-human studies, indicate peripheral plasmatic levels. ^Hypogl. and **Hypogl. indicate conditional presence/hyperglycaemia, or absence of glucose/hypoglycaemia. ^A (LCA) lithocholic acid. ^B (INT-777) semisynthetic bile acid. (GSIS) Glucose-stimulated insulin secretion. Δ(Compounds A and B) are experimental GPR119 agonists described by Li et al. (51).*

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In healthy fasted individuals, it is recognized that peripheral plasmatic active GLP-1 (7-36)\textsubscript{NH\textsubscript{2}} plasmatic levels hover around 5 pM, but within 5–10 min after an oral glucose load, they start to rise, up to a maximum of less than 10 pM after 40–90 min, and slowly descend back to baseline values in 150 min. On the other hand, the cleaved GLP-1 (9-36)\textsubscript{NH\textsubscript{2}} summed to the GLP-1 (7-36)\textsubscript{NH\textsubscript{2}} to give what is normally referred to as total GLP-1 levels, raise up to more than 40–60 pM (108). In perspective, GIP and Insulin show much broader dynamic ranges, with post meal levels reaching 300 and 400 pM respectively, from their baselines <20 pM within 30 min post glucose ingestion (108, 145). Curiously, some bariatric RYGB patients experience up to a 10-fold increase in post-meal active GLP-1 plasmatic levels (from fasting 5 pM to post-prandial 30–65 pM) (146), and have a 2- to 3-fold higher glucose-stimulated Insulin secretion (147), which in some diabetic patients results in GLP-1-mediated hyperinsulimemic hypoglycaemia that requires GLP-1 antagonism or surgical reversal of the intestinal anatomy (148).

Different authors consider the success of surgical intervention a consequence of a major change in gut hormonal profile, primarily a supra physiological post-prandial GLP-1 secretion (29, 30). This reasoning fits with the observation that type 2 diabetic patients display a shorter post-prandial peak of GLP-1, hence they are deficient for the longer response seen in healthy individuals. Multiple groups describe diabetic patients with lower plasmatic active GLP-1 but heightened GIP levels and β-cell resistance to the stimulatory effect of both GLP-1 and GIP (18, 149–153).

Nonetheless, different animal models deficient for GLP-1 signaling, in addition to human studies, prove the dispensability of GLP-1 for surgical success (31–34), questioning the causative nature of GLP-1 for the reported metabolic benefits.

On the other hand, PYY has been proven to be upregulated, and necessary, for RYGB mediated restoration of the diabetic islets, and overall cure of diabetes in rats (35) and humans (154).

Another important source of endogenous GLP-1 is the brain, a tissue where it acts as a neurotransmitter. Indeed central GLP-1 production appears essential, since peripheral GLP-1 is assumed to not be able to cross the blood-brain barrier (BBB). In particular, neurons of the hindbrain found in the nucleus-tractus solitarius (NTS) secrete GLP-1 and activate hypothalamic neurons of the paraventricular nucleus (PVN), resulting in satiety (155, 156). Indeed it is clear that PC1/3 dominant neurons of the NTS express also other the PG peptides oxyntomodulin, glicentin, and GLP-2 together with GLP-1 (157). Although expressed at much lower levels, PC2 activity has also been recognized in these neurons, and traces amounts of glucagon might have important implications.

NTS neurons-derived GLP-1 appears to reach out to multiple locations within the central nervous system (CNS), which have been proven to express the receptor, and be activated after a central administration of GLP-1 receptor agonists. These areas include the NTS itself, the suprachiasmatic nuclei, the arcuate nucleus (ARC) and the area postrema (AP) other than corticotropin-releasing hormone (CRH) PVN neurons (158, 159). Beyond satiety, this signaling appears to be a key factor for neuroprotection (160) insulin sensitivity and glucose metabolism (158).

Curiously, the feeling of satiety, is also achieved by another neurotransmitter, the Cocaine- and amphetamine-regulated transcript (CART) (161). This peptide, acts also as a hormone, and is expressed by both β-cells and intestinal GLP-1 and GIP producing cells causing GLP-1 secretion in vivo via a yet unknown GPCR (162).

It is not entirely clear to what extent endogenous GLP-1 activates all the reported GLP-1 receptor expressing neurons and to what extent it depends on the CART peptide especially in type 2 diabetes or obesity. Nonetheless, some commercial mimics of GLP-1, such as Liraglutide, even when administered peripherally, appear to cross the BBB and activate neurons within the ARC resulting in GABA dependent inhibition of neuropeptide Y (NPY) and agouti-related peptide (AgRP) secretion. This signaling has proven to be essential for the Liraglutide mediated weight loss in rats (163). GLP-1R expressing hypothalamic neurons have proven dispensable for the beneficial metabolic activity of both BBB permeable Liraglutide and Exendin-4 (164).

Singularly, BBB impermeable mimics of GLP-1 have still shown to activate GLP-1 Receptor expressing neurons (165), but they require a functional gut-brain axis through the vagus nerve (166). In particular, vagal afferent neurons expressing the GLP-1R are necessary for GLP-1 mediated induction of satiety (167) but not glucose lowering effects (168).

The complex inter-organ pharmacokinetic of GLP-1, compounds into a convoluted pharmacodynamics encompassing multiple metabolic systems.

Indeed the GLP-1(7-36)\textsubscript{NH\textsubscript{2}} receptor, a GPCR, is found to be expressed by a wide range of tissues and cells such as: α, β, and δ-cells (169), sinoatrial node myocytes, arterial smooth muscle cells of lungs and kidneys, megakaryocytes, macrophages, monocytes, lymphocytes, gastrointestinal tract mucosa [mainly Brunner's gland in the duodenum, but also in the parietal cells of the stomach, jejunum ileum and the nerve plexus around the small and large intestine (170, 171)], central nervous system [neocortex, cerebellum, thalamus, amygdala, area postrema, hypothalamus, hippocampus, nucleus tractus solitarius (158)], peripheral nervous system (myenteric plexus) and in the skin (14, 172–176).

Counterintuitively, mice completely defective for the GLP-1 receptor were reported to be protected from high-fat diet-induced peripheral insulin resistance (177) and, consistently with this, central inhibition of GLP-1R signaling with the antagonist exendin 9-39 improves glucose tolerance and glycaemia (178). Conversely, mice defective for both the receptors for glucagon and GLP-1, or GLP-1 and GIP, show a highly plastic entero-pancreatic system that adapts and gives these animals no overt phenotype in terms of glucose homeostasis (105).

Nonetheless, the pharmacological activation of the GLP-1R is clinically beneficial (179), offering an improved glycaemic control with lower cardiovascular morbidity and without the risk of hypoglycaemia associated with some current anti diabetic drugs (173). Furthermore, being an appetite suppressant, GLP-1 signaling also helps to lose body weight, especially if in combination with metformin. Conversely, anti-diabetic drugs such as sulfonylureas, or Insulin, are known to induce not only weight gain (180, 181), but also an increased risk of
hypoglycaemic events (182). Pharmacological activation of the GLP-1 Receptor has also shown to help exogenous insulin in the control of glycaemia in patients with type 1 diabetes, by slowing the gastric emptying and blocking glucagon secretion (183, 184).

Currently, six different peptide GLP1-Receptor agonists are on the market, with more in clinical trials. In particular, two short-acting formulations of Lixisenatide and Exenatide and four long acting preparations of Exenatide, Lisproglutide, Dulaglutide and the most recent and successful Semaglutide, were approved in October, 2017 for the North American markets by FDA (25, 185). The first GLP-1 analog to be approved by FDA in 2005 for the management of Type 2 diabetes was the chemically synthesized Exenatide under the name of Byetta (186), a formulation of the DPP-IV resistant peptide discovered in the gila monster Heloderma suspectum saliva in 1992 (187). Despite the longer half-life in serum, Byetta needs to be injected twice a day. In the last decade, formulations with extended release entered the market with once-weekly self-administrations pens.

Pleiotropic beneficial effects have been reported for this class of drugs. Beyond the improved glycaemia control, essential for the short term treatment of diabetes (188), different GLP-1RAs are powerful clinical tools for the management of diabetic kidney disease (DKD) (28, 189) non-alcoholic steatohepatitis (NASH) (190), neuroinflammation (191), obesity and cardiovascular disease (192–195).

Although GLP-1RA are improving the lives of patients affected by type 2 diabetes or the metabolic syndrome (196), the physiology of GLP-1 is far from being clear.

More recent data suggest how the unimolecular co-activation of GLP-1 and GIP receptors, has powerful anti-diabetic effects superior to either agonism (197). Furthermore, oxyntomodulin is a natural dual-agonist of GLP-1 and glucagon receptors and displays anti-diabetic properties in humans (198, 199). Upon this finding, a tri-agonist peptide, targeting the receptors of GLP-1, GIP, and glucagon was created (200). The in vivo effects of this drug are unparalleled, even superior to what can be achieved with the dual agonists for either combination. The synergistic activation of these three important receptors is capable to revert diet-induced obesity, cognitive impairment and T2D in mice models, warranting future human studies (201, 202).

**EXPANDING THE PHYSIOLOGY OF GLP-1**

When examining the physiology of glucagon-like peptide-1, it is important to consider that there is an expanding body of evidence that questions its systemic endocrine physiology (203, 204). Pancreatic α-cells have been demonstrated to express and secrete not only GLP-1 (205, 206), but also PYY (35) GIP (207, 208) mini-glucagon (209) or even Xenin (210) together with glucagon (Figure 2). The key protease responsible for the processing of the proglucagon peptide into GLP-1 is Pcsk1/3, which has been shown to be upregulated in α-cells during hyperglycaemic, hyperlipidemic, or inflammatory conditions to promote glucose-induced glucagon suppression, a compensatory response to a metabolic insult as in type 2 diabetes (205). Insulin itself has shown to modulate PCI/3 expression to possibly aid its own metabolic activity (211).

Recently, the whole dogma of the role of intestinal GLP-1, envisioning the traveling from the gut to the liver and ultimately reaching the pancreatic β-cells to bind its GLP-1R has been questioned in transgenic mice (204). Indeed, since both DPP-IV degrades and NEP24.11 degrade GLP-1 within seconds, the possibilities of any intestinal GLP-1 to reach the system circulation and then the islet microcirculation are doubted. Besides, it is important to consider that intestinal GLP-1 has a local concentration in the nM range (10–100 pico moles per gram of tissue, see Figure 1), further advocating that the main action of this protein has evolved to be locally restricted.

Animals deficient for the GCG gene in the intestine, still experience a normal incretin effect disrupted with the GLP-1R antagonist Exendin (9-39) (204). This indicates that it is the intra islet, α-cell derived GLP-1 that shows the meal-induced insulinoergic properties. A critic to the use of a murine model deficient for intestinal GCG products, would be that other gut hormones might compensate for the lack of a functional GCG gene in that tissue, hence explaining the normalized incretin effect. Indeed other gut hormones such as GIP must be responsible for the incretin effect to a higher degree than once thought. Nonetheless, it is also clear that intra-islet GLP-1R signaling is essential for GSIS, with more evidence that an intra-islet paracrine GLP-1 signaling is physiologically present (212, 213) and necessary for β-cell health under metabolic (214).

In contrast, mice deficient for GLP-1R only in β-cells have a normal incretin response and oral glucose tolerance, indicating the dispensability of intra-islet signaling of GLP-1 for the incretin effect. Interestingly, these same animals have an improvement of their glucose tolerance in response to oral DPP-IV treatment, but not to subcutaneous GLP-1 mimics, indicating how the former relies completely on localized, non β-cell GLP-1R (215).

There are still multiple gaps into our understanding of how different GLP-1 producing tissues communicate, especially in the brain to islet axis. It is known that acute, but not chronic, central GLP-1 receptor activation directly modulates glucose-induced Insulin secretion implicating a direct brain to islet neuronal communication (61).

On the other hand, chronic GLP-1 activity in α-cells increases its own secretion, feeding an autocrine loop that gets overstimulated with the use of exogenous synthetic GLP-1R agonists (198; Figure 2). Curiously in diabetic rats, it has recently been shown that this loop might indeed induce the production of more glucagon than in healthy animals (99).

It has been known for more than two decades and has been confirmed more recently, that an infusion of GLP-1(7-36)]N$_\text{H}_2$ has insulinoergic and glucagonostatic effects. This is seen when the plasmatic levels are above 50–60 pM, equivalent to more than five times the levels seen post-prandial in healthy individuals challenged with a bolus of glucose, or 10-fold their basal levels (153, 216), adding further doubt to the physiological hormonal dogma of intestinal GLP-1. Considering the mounting

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1. http://press.novonordisk-us.com/2017-12-5-Novo-Nordisk-Receives-FDA-Approval-of-OZEMPIC-R-semaglutide-Injection-For-the-Treatment-of-Adults-with-Type-2-Diabetes
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FIGURE 2 | The gut-brain-islet axes of GLP-1. The intestinal EECs secretome is subject to first pass metabolism, while intraislet signaling relies on paracrine signaling. Intestinal cells are known to communicate with the Enteric Nervous System, and the Central Nervous System through the Vagus Nerve. Neuronal engagement between the gut lumen and the islets of Langerhans is a possible compounding explanation to the incretin effect, whereby the mechanism of the single molecular players are still largely unknown. See text for further details.

In addition, both in vitro and in vivo Interleukin-6 (IL-6) has shown to be a powerful GLP-1 secretagogue, capable to positively modulate both the proglucagon gene, and the expression of PC1/3 in α-cells and intestinal L-cells (225, 226). Indeed, GIP has shown to not only be co-expressed with GLP-1 and glucagon in α-cells (207); it also stimulates in an autocrine/paracrine fashion the expression of IL-6 in the same α-cells, thus indirectly acting as a GLP-1 secretagogue (95).
IL-6 has shown also to induce the secretion of intestinal GLP-1, indirectly via the release of adipocytes derived Leptin (227).

Curiously, it was recently reported that this pro-inflammatory cytokine, IL-6, similarly, but independently from GLP-1, slows gastric emptying (228). Furthermore an inflammatory status, as seen in pathologies such as type 2 diabetes, might compromise the gut mucosal permeability, leading to the exposure of intestinal EECs to luminal LPS, and a TLR4-mediated release of GLP-1 (229). This is consistent with the knowledge that GLP-1, as well as glucagon, has shown to possess powerful anti-inflammatory properties in vivo, an area that hold with vast therapeutic potential (136, 230).

Ghrelin is another possible player, since it has been proven to be expressed not only in the gut, but also in a distinct subpopulation of islet cells named ε-cells (231) and, being known to be a stress-induced (232) GLP-1 secretagogue (233, 234), it might play an important role in the intra-islet signaling.

Recently, it has been demonstrated that mice with a deletion of the GLP-1 receptor only in β-cells, are resistant to the beneficial anti-diabetic effect of a vertical sleeve-gastrectomy (36), suggesting how GLP-1 activity in β-cells is key to the bariatric surgery success. It is not known if intra-islet α-cells production of GLP-1 is affected by the surgical procedure or, more importantly, how this axis is impaired in the metabolic syndrome, type 2 diabetes and related pathologies.

It appears that only in RYGB and SG patients intestinal derived GLP-1 has a true endocrine role, while in healthy individuals, localized, paracrine and neuronal signals primarily define the GLP-1 physiology.

It is therefore clear that currently available GLP-1RAs, mimicking on the peripheral action of GLP-1 (7-36)NH2, not only ignore the yet unknown physiology of GLP-1 (9-36)NH2 or its metabolites, but they also fail to address the tissue specific physiology of GLP-1 (7-36)NH2, while pushing to supra-physiological limits the endocrine GLP-1 receptor axis, likely explaining the reported side-effects and only partial success in the treatment of T2D.

In addition, it is important to notice that the ubiquitous DPP-IV protease targets not only GLP-1 but also oxyntomodulin, GIP and PYY among other proteins (235). Specifically, the GLP-1 co-secreted cousin PYY(1-36), agonist of the vasoconstrictive Y(1) receptor, is physiologically trimmed by DPP-IV to give rise to the appetite-suppressant, anti-diabetic and blood-brain barrier permeable PYY(3-36) agonist of Y(2) receptor (220). It is therefore clear that pharmacological DPP-IV blockage disrupts this axis and induces hypertension (236).

Recent studies provide new evidence supporting the paracrine nature of intestinal GLP-1, whereby Serotonin-(5-HT)-secreting enterochromaffin (EC) cells are directly stimulated by locally produced GLP-1, which in turn stimulate afferent Vagal nerves (Figure 2) bridging the gut to brain axis. Accumulating evidence suggest that, especially in the colon, EC cells express multiple receptors for the microbiome metabolites, representing a new important link bridging the microbiome to the brain (237, 238).

A better way to amend the pathophysiology of GLP-1 reported in diabetes or other diseases, would be to induce tissue specific de novo GLP-1 production, leading to a more physiological and likely safer, short and medium distance signaling. Numerous attempts have been made with multiple GLP-1 secretagogues such as GPR119 agonists (239) but so far no compound has reached the market because of bioavailability issues and systemic off-target toxicity. One possible way to minimize the side-effects of the single drugs is to combine them to achieve synergistic effects, as reported recently with a combination of a DPP-IV inhibition, SSTR5 antagonism and GPR40 and TGR5 agonism, capable to raise circulatory active GLP-1(7-36)NH2 levels to more than 300-400 pM in mice (240).

### SWEETNESS IN THE GUT

Studies in vitro and ex-vivo with isolated human primary cells suggest that there are two temporally distinct pathways that lead to the glucose-stimulated release of GLP-1, similarly to what happens in β-cells with the 1st or 2nd phase insulin release. A quick mechanism independent of the cell energetical state and a slower one, metabolism dependent, mediate the release of this incretin (53, 72).

The 1st phase in the pathway of glucose signaling, sees the electrogenic sodium-coupled glucose transporters 1 (SGLT1) mediated uptake of two Na+ ions for every internalized glucose molecule (53). This depolarization is propagated through voltage-dependent Calcium and Sodium channels, which currents lead to the discharge of the hormones containing vesicles (72).

The 2nd phase is exemplified by the absorption of simple sugars, such as Glucose or Fructose, via the facilitative transporters GLUT2 and GLUT5 respectively, which leads to an increased internal metabolism mirrored by intracellular ATP levels. This state leads to the blockage of ATP dependent potassium channels and the subsequent membrane depolarization, followed by the secretion of the hormonal cargo.

Mace et al. (241) demonstrated how diazoxide, a K+ ATP channel opener, completely abolished the glucose-dependent incretin release while a channel blocker, tolbutamide, exacerbates it in terms of secreted GLP-1, GIP and PYY.

More recent data, question the first mechanism in enteroendocrine cells. Glucose mediated GLP-1 release happens in humans only in the proximal and distal small intestine and independently of ATP mediated potassium channels closure. Furthermore, concentrations of up to 300 mM glucose do not induce GLP-1 secretion from colonic human mucosa because GLP-1 producing L-cells barely express SGLT1 (43, 53, 58, 72).

Consistently, the use of α-methyl-D-glucopyranoside (MDG), an acoloric substrate of SGLT1, within 5 min triggers the release of GLP-1 as glucose does, demonstrating how it is the sodium current that triggers the release of the incretin, and not the metabolic ATP-driven arrest of potassium currents and following calcium spike (58).

The pharmacological blockage of SGLT-1 with phloridzin, in a rat small intestine perfused system, results in just a halved secretion of GIP, GLP-1, or PYY, and the addition of phloretin,
a GLUT2 inhibitor, brings these values down to basal levels. In fact, this double blockage of SGLT1 and GLUT2, completely inhibits the responsiveness to other stimulants as well, such as sucralose, glycyrrhizin, OEA, propionate and taurocholate. The activity of the calcium channel CasR is also essential for the responsiveness to free amino acids (241).

All these observations are challenged by longer term in vivo studies. Blockage of SGLT-1 markedly improves glucose-stimulated GLP-1 release if a 3-h long period is considered.

The rationale given by Oguma et al. (242) is that SGLT-1 is expressed mainly in the small intestine, hence its inactivation results in heightened luminal glucose that travels down to the colon where it someway stimulates GLP-1 release. Given the fact that SGLT-1 is barely detectable in colonic proglucagon positive cells and that potassium channels in this tissue are unresponsive to sulfonyureas, the molecular sensor(s) that causes the release of GLP-1 in vivo, remains elusive.

Another enigmatic G protein is α-gustducin, a key element in sweet-taste transduction pathways downstream of the heterodimer formed between the GPCRs Tas1R2 (T1R2) and Tas1R3 (T1R3).

Its expression has been reported in colonic L-cells and appears to be responsible for the glucose-stimulated release of incretins (243, 244). This is confirmed by the impaired glucose-stimulated release of GLP-1 in mice lacking either T1R3 or α-gustducin (244).

Interestingly, this axis is also activated by the disaccharide sucrose and by the non-metabolizable and therefore anergic sucralose (243). Of note also Aspartame, Acesulfame K, Glycyrrhizin and Saccharin bind the sweet receptor heterodimer Tas1R2/3 and they have shown to stimulate GLP-1 secretion in the human duodenal adenocarcinoma-derived HuTu-80 cell line (245, 246). Despite this report, other groups weren’t able to replicate these results (243). Indeed, it was shown that proglucagon expressing cells, derived from the colon of Venus mice cultures, were not responding significantly to Sucralose (1 mM) in terms of both released GLP-1 and intracellular Calcium. Conversely, proglucagon negative cells responded to the sweeter. More doubts about the role of Tas1 receptors were raised after the demonstration that oral gavage with sucralose, saccharin, stevia, acesulfame potassium or tryptophan do not cause a gut incretin release in Zucker diabetic fatty rats (247).

### LONG AND MIDDLE CHAIN FATTY ACID RECEPTORS

The study of the receptome of enterendocrine cells, has provided invaluable pharmacological insight with the discovery of proteins capable to sense multiple compounds once thought to be only nutrients.

A prime example is given by two GPCRs, GPR40 and GPR120, also known as Free Fatty Acid Receptor 1 (FFAR1) and 4 (FFAR4) respectively. These chemosensors are two major molecular players in the detection of dietary, medium (C8-12) and long (C14-22) chain fatty acids (LCFA) (84, 248).

GPR40 is primarily expressed by the pancreatic β-cells, where it plays a pivotal role in FFA-mediated lipotoxicity (249) but also in α-cells (78, 250), CCK (80), GIP (251), and GLP-1 (79) producing cells in the gut and in hypothalamic neurons (248, 252, 253). Animals deficient for this receptor are protected from obesity-induced hepatic steatosis, hyperinsulinemia, hypertriglyceridemia and hyperglycaemia. More than a decade ago a study showed that GPR40 mediates the long-term FFA-induced lipotoxicity seen in the diabetic islets (254); nonetheless, these findings are still under debate today. Recent data are still highly polarized, with some authors supporting (255), and others disproving this (256), or even indicating that GPR40 protects β-cells from lipotoxicity (257) rendering difficult to draw any conclusive mechanistic involvement in healthy and diabetic individuals. Nonetheless, the activation of this receptor with FFAs has demonstrated to induce the secretion of incretins (79, 258) glucagon (78, 250) and partially glucose-stimulated insulin (259, 260) reducing food intake, and lowering body weight in animals models (261). Mice without a functional GPR40 display an impaired CCK and GLP-1 secretion after an oil gavage, while surprisingly animals deficient for GPR120 display a normal corn oil-induced GLP-1 secretion (80, 262).

GPR40 is coupled to both Gq and Gs proteins and in vivo studies suggest how signaling through both these cascades elicits the most powerful GLP-1 secretion (258). Ligands that bind GPR40 and activate predominantly only the Gq pathway are not good GLP-1 secretagogues. Indeed recently it has been shown that dietary triglycerides appear to induce the secretion of GLP-1 via GPR40 in synergy with the Gs activating GPR119 (263). Nonetheless, chylomicrons have been reported to be powerful GPR40-Gq activators and GLP-1 secretagogues, acting from the basolateral side of the intestinal mucosa (264).

The two synthetic GPR40-specific compounds AM-1638 and AM-5262, have been found to act as double Gq and Gs agonists but also as positive allosteric modulators, capable to enhance the GLP-1-secreting capabilities of Gq-only agonists such as dietary docosahexaenoic (DHA) and α-linolenic acid (ALA), independently of the orthosteric site (265).

GPR120 shows very little sequence similarities to the other free fatty acid receptors but, likewise, is found to be expressed by the enteroendocrine cell system, especially in the colon (see Figure 3), but also in the lungs (267), white and brown adipose tissue (274, 275), hypothalamic microglia (253), macrophages and, contrarily to GPR40, not in β-cells but in somatostatin producing δ-cells (276). Both small intestinal GIP and colonic GLP-1 secreting cells express GPR120, and the molecular cascade triggered by this receptor has been shown to mediate dietary incretin release directly or indirectly through CCK (84–86). Interestingly, both of these two receptors are expressed only by a fraction of hormone positive EECs; in particular, it has been reported that only 3% of GLP-1 positive cells express GPR40, and 23% GPR120 (266).

GPR120 displays a ligand preference similar to GPR40; a broad range of long chain fatty acids signal through it, with some ligands eliciting more robust calcium responses than others (84). Multiple dietary compounds have shown to be powerful agonists of GPR120, such as pinolenic acid, a poly-unsaturated fatty acid...
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**FIGURE 3 | Gastrointestinal GLP-1-secreting receptome distribution.** Summary of available expression studies of different GLP-1-secreting receptors along the gastrointestinal tract. (A) GPR40/FFAR1 has been reported to be expressed in the small intestine in different EECs, with overall higher transcript levels than GPR120, and superior co-localization with GIP in the distal small intestine (79, 80, 266). (B) GPR120/FFAR4 has shown co-expression with both proximal small intestinal GIP+ and large intestinal GLP-1+ cells (85, 266–268). (C) GPR43/FFAR2 and (D) GPR41/FFAR3 are co-expressed by all types of enteroendocrine cells, from the stomach to the rectum, especially in the colon (83, 269, 270). (E) Reports of comparative GPR119 transcript are contradictory, while immunohistochemical data indicate co-localization with a minor fraction of CCK and GLP-1 positive cells mainly in the stomach and small intestine (266, 271). (F) TGR5, has been reported equally distributed along the whole gastrointestinal tract of dogs (101, 272, 273).

(C18:3 trans, cis, cis Δ 5, 9, 12) found in pine nut oil (277), or the yeast derived phytosphingosine (278).

In macrophages and adipose tissue, GPR120 mediates ω-3-mediated anti-inflammatory and insulin sensitizing effects (279, 280). Contrarily to GPR40, the genetic deficiency of GPR120 is more dramatic. Knockout animals show hyperinsulinemia and insulin resistance, hyperglycaemia and osteoarthritis (281), hepatic steatosis and therefore obesity. Furthermore, an absence of GPR120, results in an overactive glucagon signaling, explaining the hyperglycaemia (282). Indeed, in humans, a single aminoacid mutation of the receptor that hampers its signaling is associated with obesity and insulin resistance (283). Expectedly, GPR120 agonism shows powerful anti-diabetic, anorexic, and hepatoprotective properties in multiple animal models (275, 284–287), at least partially mediated by GLP-1 (288).

Considering the overlap of natural ligands of GPR40 and GPR120, it has been difficult to study them individually and understand their individual physiology, while recent data indicate that indeed these two receptors work synergistically, to exert anti-diabetic activity in vivo from the gut (289), and the brain (253).

Despite these advancements, in clinics there are currently no available drugs targeting GPR40 and GPR120. TAK-875, the best candidate for GPR40 which showed promising GSIS capabilities up to Clinical Phase III for the treatment of T2D, had to be halted because of hepatotoxicity and alteration of bile salts composition (290).

Despite these setbacks, encouraging animal data warrant future efforts for the development of new drugs capable to activate synergistically both GPR40 and GPR120 and mediate, through GLP-1 and other intestinal, pancreatic and cerebral peptides, better treatments for multifactorial chronic metabolic diseases.

**SHORT CHAIN FATTY ACID RECEPTORS**

In 1997, four 7 α-helixes transmembrane receptors, GPR 40, 41, 42, and 43 were mapped on the same locus found on the long arm of chromosome 19 (291). Soon after, different groups identified GPR 43 and 41 as the receptors for free fatty acids, which were then chronologically renamed FFAR2 and FFAR3 respectively (292–294).

Both these receptors are activated by similar types of short chain fatty acids (292), and both these signal through an inhibitory G type protein, but FFAR2 is also capable to signal...
through Gq/11 proteins (293) by which it has shown to mediate GLP-1 and PYY secretion in *vitro* and *in vivo* (82, 295).

Along the gastrointestinal tract, both GPR 41 and 43 have been reported to be co-expressed, with FFAR2/FFAR4 at higher levels and overall number of cells, especially intraepithelial leukocytes, while FFAR3/FFAR4 is found on submucosal neurons [see Figure 3, (83, 295–297)]. Indeed FFAR2 holds promise for the management of Inflammatory Bowel Disease (IBD) (298) a possible side-effect of anti-diabetic treatment with DPP-IV inhibitors (299).

Feeding rats with fructo-oligosaccharide as a source of SCFAs has also shown to upregulate FFAR2 (270). Recently, both the receptors have been shown to heteromerize in *vitro*, eliciting synergistic signaling and β-arrestin-2 recruitment (300). Furthermore FFAR2 activation in *vitro* with an inulin-enriched diet in mice results in PYY release and proliferation of L cells in *vitro* (81). Nonetheless, there is still some controversy on the in *vivo* involvement of FFAR2 and FFAR3 in GLP-1 modulation (301, 302), with some reports indicating that blockade of GPR43 in *vitro* releases GLP-1 (303) and others indicating different mechanisms of action, with FFAR2 releasing PYY from intestinal L-cells (81), while FFAR3 restricted to submucosal neuronal activity (295) despite its apparent expression by the majority of enteroendocrine cells (83).

In pancreatic β-cells, both GPR43 and GPR41 are expressed, and the latter antagonizes GSIS (304).

Adding complexity to the study of these receptors, there is extensive species-specificity, so that animal findings result in poorly translatable data, requiring the generation of complex human-murine chimera currently under intense study (305, 306).

Nonetheless, considering that the half-maximal effective concentration (EC50) for Acetate, Propionate, and Butyrate is around 0.5 millimolar upon both GPR41 and GPR43 (292) and that the SCFA concentration in the human ileum and colon lumen is superior to 100 millimoles per kg (307–309), it is likely that both receptors are constitutively active. Obese patients, have been reported to produce more SCFAs in their intestines (310), but indeed meaningful diet-induced shifts in SCFA production fluxes have proven not sufficient to modulate peripheral levels of GLP-1 and PYY (311).

GPR42 is another G-Protein-Coupled-Receptor that was initially considered to be an inactive pseudogene derived from GPR41. In 2009, 29% of 202 human alleles of GPR42 were shown to have an inactivating single nucleotide polymorphism (SNP) at W174, and 61% with an arginine in like GPR41, resulting in a fully functional receptor, differing from it by only 5 aminoacids (312). A more recent study highlights how GPR42 is not only functional, but displays a pool of haplotypes in a great proportion of humans, with a distinct pharmacology (313).

**GPR119**

GPR119, also known among other names as glucose-dependent insulinotropic receptor (GDIR), was independently discovered less than two decades ago by several groups around the world and deorphanized soon after with the discovery of Oleoylthanolamide (OEA) as its first endogenous ligand (314–316).

Recently our group has demonstrated that indeed OEA is just a partial agonist of GPR119, and the biological ligand of this receptor is the lysophospholipid Oleoyl-Lysophosphatidylinositol (Oleoyl-LPI) (87). This bioactive lipid induces a powerful GPR119 mediated-GLP-1 secretion *in vitro* and *ex-vivo* from intestines of wild type, but not GPR119 deficient mice. This peculiarity is not shared by LPI species with different aliphatic chains, which have been described as the ligands of GPR55 (317).

This GPCR is primarily expressed in the pancreas by α-cells, β-cells and γ-cells (271, 318, 319), and is found at lower concentrations along the GI tract, especially in the stomach and duodenum, where counterintuitively only a minor fraction of CCK, and GLP-1 expressing duodenal enteroendocrine cells display GPR119 (266, 271). This receptor is also expressed, and hence can be studied, *in vitro*, by the human enteroendocrine cell model NCI-H716 or by the murine GLUTag cell line (320). Heterologous expression in *vitro* unveiled its constitutive activity capable to raise intracellular cAMP levels through Gαs (321) and lead to the secretion of GLP-1 and PYY (89). Rodents, contrarily to humans, express GPR119 also in some regions of the brain (316). The activation of this receptor is known to mediate glucose-stimulated insulin secretion and a glucose-independent release of incretin hormones by intestinal enteroendocrine cells (88).

Long-chain fatty acids and phospholipids like lysophosphatidylcholine (LPC), other compounds such as retinoic acid (RA) and multiple N-acylethanolamines (NAE) such as N-oleyl dopamine (OLDA), palmitoylethanolamide (PEA), or oleoylthanolamide (OEA), all act as endogenous ligands of GPR119. OEA is a more potent GPR119 agonist than its glycerol ester 2-Oleoyl Glycerol (2-OG) found in olive oil (322).

Indeed, oleic acid is internalized via CD36 and converted to OEA in the duodeno-jejunal enterocytes, which in turn causes satiety directly via PPAR-α (323) or indirectly through an incretin secretion mediated via GPR119 in the gut (324). Curiously, fat-induced OEA synthesis is a fairly conserved pathway in metazoan, being present in fish and extremely slow-metabolism reptiles such as pythons (325, 326).

Triglycerides, with medium length fatty acids such as 1,3 Diocanoyl- 2 Oleoyl glycerol, can also cause the release of GLP-1 in humans. However, this happens via the metabolized 2-OG component, since dietary medium chain fatty acid do not cause any appreciable release of incretins (323).

Counterintuitively, long term olive oil feeding does not improve glucose tolerance or insulin responses in diabetic rats (5). Indeed, more recently it has been reported that a high-fat diet enriched in oleic acid leads to an impaired endogenous OEA and other N-acyethanolamides intestinal production in mice (327), suggesting that a chronically resistance is taking place within the OEA synthesis pathway.

Surprisingly, a daily activation of GPR119 with OEA or other synthetic agonists, increases β-cell responsiveness in islets transplanted into STZ-induced diabetic mice (328).
The importance of GPR119 in the fat-induced incretin secretion is demonstrated by the impaired incretin signaling displayed by transgenic animals deficient for this protein only in PG expressing intestinal cells. Male and female mice, completely loose the GLP-1 response to an oral gavage of olive and corn oil (329).

More recently, it was reported that whole-body GPR119−/− knockout mice are protected from high-fat induced glucose intolerance and insulin insensitivity. Interestingly, the specific ablation of GPR119 only in β-cells does not affect glucose tolerance nor insulin secretion. In fact AR231453, a selective GPR119 agonist, improves glucose tolerance and insulin sensitivity in both WT and Gpr119−/− mice, suggesting how insulin release is independent from pancreatic GPR119 but depends on gut incretin release (330).

Curiously, GPR119 activity appears to be directly dependent on the PYY receptor NPY1 (331). This phenomenon is independent of DPP-IV, the GLP-1 receptor, or the PYY related peptide NPY.

Furthermore, GPR40 also shows synergism with GPR119, mediating a more than additive GLP-1 response to triglycerides in the large intestine (263).

Agonism of GPR119 in both healthy or diabetic and obese mice, is known to improve glucose tolerance (90), or even prevent atherosclerosis in mice (332), while at the same time inducing the secretion of glucagon under low glucose levels avoiding hypoglycaemia (91); therefore since 2008, multiple agonists have been synthesized (239, 254, 333), as well as unimolecular dual DPP-4 inhibitors and GPR119 agonists (334). Despite the good results seen in rodents, species-specific pharmacology might be to blame (335).

Up to now all the prospective GPR119 agonists were plagued by low bioavailability, lack of efficacy and more importantly, cardiotoxicity which has stopped all human studies before any large scale Phase III clinical trials (239).

Despite the multiple failures, the compound DS-8500a is showing promising glucose lowering properties in Phase II clinical trials without any apparent toxicological issues in clinical trials (92).

**TGR5**

Bile acids (BAs) are cholesterol-derived molecules produced in the liver and temporarily stored in the gallbladder. When food is ingested, BAs are released into duodenum to solubilize dietary lipids under the form of micelles, a necessary step for the maximization of the surface-volume ratio of fat droplets, aiding interface-acting lipases.

Indeed the release of lipids from micelles has directly proven to release GLP-1 and GIP via the FFA1 in the duodenum (264).

This release of bile acids, mainly cholic (CA) and chenodeoxycholic (CDCA) acid derivatives, happens through the relaxation of the smooth muscle sphincter upon CCK signaling (336) or indirectly through a similar VIP action on the sphincter of Oddi (337).

Historically described as mere fat-solubilizing agents, these amphipathic compounds were recently recognized as key signaling molecules capable to modulate the host metabolism directly acting as ligands of intestinal GPCRs (101, 338, 339), or after being metabolized by the colonic microbiota into secondary bile acids, mostly deoxycholic and lithocholic acid (340).

The chemosensor believed to be the main receptor of bile acids is TGR5, also known as GPR131 or GPBAR1 among other names. This receptor has been reported to be expressed by colonic GLP-1-secreting enteroendocrine cells and pancreatic α- and β-cells, with some controversy regarding the presence in murine islets (339).

TGR5 activity appears to not have been lost in type 2 diabetic humans whereby the infusion of CCK, or rectal taurocholate, causes GLP-1 and insulin release via the TGR5 axis in colonic L-cells and pancreatic β-cells respectively (341, 342).

This notion is in stark contrast to the well-known anti-diabetic properties of BAs sequestrants, (343) and some, have proven to elicit GLP-1 secretion via TGR5 mediated PC1/3 upregulation (344). A likely explanation is that the BAs bound to a sequestrant into the intestinal lumen can’t be absorbed and hence travel more distally in the GI tract where the complexes are still capable to activate the TGR5 expressing colonic L-cells. Furthermore, the lower systemic levels of bile salts prompt the liver to produce more bile, which in turn feeds more TGR5 agonism into the colon (343).

This chemosensor is expressed by the pancreatic α-cells where its signaling activates Gs proteins and induces the secretion of GLP-1 directly through Epac proteins and indirectly via CREB mediated expression of Psck1, while in β-cells mediates insulin release ([100]; Figure 3).

TGR5 is the target of different BAs, but the most potent endogenous agonist has shown to be lithocholic acid (LCA) and its taurine conjugates with activity at nanomolar concentrations (273, 339). Secondary bile salts, metabolized by the microbiota, exhibit less potency toward this receptor.

Despite this promising anti-diabetic activity of TGR5 mediate by GLP-1 (345), its pharmacological activation in diabetic patients has shown side effects at the level of gallbladder and heart, hampering its clinical use (346).

Another bile salts chemosensor is the nuclear farnesoid X receptor (FXR) (347) which activation, contrarily to TGR5, blocks the release of GLP-1 in the colonic L-cells (348), while in the liver induces glycogenesis helping to improve glucose homeostasis. This counterintuitive pharmacology has been confirmed in vivo whereby the administration of the FXR agonist GW4064 by mouth drives hyperglycaemia and obesity (349) while intraperitoneal injection exerts protection from it (350). Consistently, an indirect inhibition of intestinal FXR through microbiota modulation, or genetic deletion of intestinal FXR, corroborate this phenomenon displaying protection from high-fat diets induced obesity and fatty liver disease (351).

This could explain why bile acid sequestrants support a positive glucometabolic homeostasis. Indeed, the insoluble complexes of bile salts can activate lumen-facing TGR5 receptors, while they cannot cross plasma membranes to activate intracellular GLP-1-suppressant FXR receptors.

FXR is a very important receptor, part of a negative feedback in the liver, whereby the binding of bile salts, especially...
chenodeoxycholic acid, represses the de-novo synthesis of bile salts (352, 353). Indeed, there are multiple primary or secondary bile acid chemosensors in the liver (348, 354) or scattered along the gastrointestinal tract (355), where they ensure a direct negative feedback aiding detoxification (356) and protecting from hepatotoxicity and carcinogenicity displayed by some secondary bile salt such as lithocholic acid.

Accumulated evidence, indicate how bile acids are important modulators of the whole body metabolism, bridging the microbiome to the brain, likely being key signaling molecules in the pathogenesis of obesity and type 2 diabetes. Indeed remittance from diabetes experienced by RYGB or SG patients, has been attributed to the elevation of circulating bile acids (37, 38, 357), warranting further investigation, especially the development of gut-restricted TGR5 agonists (358).

TRPV1 AND THE TRP CHANNEL FAMILY

The transient receptor potential vanilloid 1 (TRPV1) is a tetrameric non-specific cationic channel found in most of mammalian sensory neurons (359). Each of its constituting monomers crosses the plasma-membrane six times and both the N and C-term face the cytoplasmic side, where they make up 70% of the receptors’ entire volume (360). This chemosensor, together with other 27 non-selective cationic channels, is part of a larger family named transient receptor potential (TRP) channel superfamily and is known to play an important role in the metabolic syndrome (361, 362).

TRPV1 is primarily activated by vanilloids and capsaicinoids including Capsaicin (360), eliciting the sensation of spiciness; multiple stress-related stimuli cause its activation and opening with subsequent membrane depolarization. For example cigarette smoke, excess of protons (pH < 5.9) (363), temperatures above 43° (360), certain animal toxins (364, 365), ATP (366) or even cannabinoids such as Anandamide (367) and cannabidiol (359, 368), are all stimuli known to activate this sensor. Indirect stimulation has also been demonstrated by bradykinin (366), NGF (366), PGE2 (369), PGII (369) and agonists of Protease-activated Receptors (PARs) (370).

TRPV1 has been shown to be expressed in the brain, β-cells (371), nociceptor C fibers, dorsal root ganglia, hepatocytes, spermatozoa (372), airway neurons (373), bladder and urothelium (374), blood vessels, and the whole gastrointestinal myenteric plexus (375), especially in colonic and rectal neurons (376). Consistently, TRPV1 is also found to be expressed by the murine enteroendocrine cell line model STC-1 and its agonism induces the release of GLP-1 in vivo (377).

This receptor has recently been seen an increasing interest since its activation has been found to have pleiotropic beneficial metabolic effects (378).

Indeed, it has been known for more than a decade that capsaicin is capable to elicit a glucose-stimulated insulin release in vivo (379). A crossover study operated on 30 human healthy subjects (380), showed a slight increase in plasmatic GLP-1 and a slight decrease in ghrelin levels 30 min after a Capsaicin enriched meal (containing 1,030 mg of 80,000 Scoville heat units red pepper); Peptide YY changes were not statistically significant. Despite these promising results, TRPV1 knockout mice display contrasting phenotypes with the report of opposite phenotypes. One author describes an obese insulin and leptin resistant mouse (381), while another group report animal protected from diet-induced obesity (382).

Considering all the recent findings, drugs targeting TRPV1 would be beneficial for the management of obesity (383) metabolic syndrome (384) and type 2 diabetes (385). Nonetheless, considering the EECs receptorome responsible for gut-peptide modulation, TRPV1 has received much less attention, with a yet largely unexplored physiology.

THE MICROBIOTA

Animals’ GI tract is known to host a population of hundreds of different species of bacteria (386), viruses and fungi, estimated to equal in number the cells that constitute the human body (387). These microorganisms thrive in the colon’s lumen, where they secrete small molecules ultimately affecting the host immunity (240) and metabolism (388).

The relative abundance of different microbial species is known to depend on the presence of specific nutrients (389); hence, considering an imbalance in the microbiota correlates with chronic inflammation pathologies of the bowel, or even Type 2 diabetes, it is likely that dietary components indirectly influence the occurrence of these pathologies via the microbiota (390, 391).

The human colonic microflora is known to produce high concentrations of Short-Chain-Fatty acids (SCFAs), among other metabolites, from the anaerobic fermentation of dietary indigestible carbohydrates, or even derivatives of bile salts (389). In fact, the SCFAs Acetate, Propionate and Butyrate are the principal luminal anions in humans and other mammalian’s colon (309, 392), with some inter-species variability. Rats show higher levels of fecal Acetate, 75 mM vs. human’s 50 mM, Propionate, 27 vs. 11 mM and Butyrate, 16 vs. 5 mM respectively. On the other hand, surprisingly similarly to humans’ colonic and fecal values, rumens of herbivores, such as sheep or cows, also contain high levels of acetate, propionate and butyrate, with reported concentrations of 65, 21, and 18 mM, respectively (308). These levels appear to be independent of dietary proteins or fibers; conversely, it is the caloric intake that affects the relative composition and concentrations of SCFAs (308). These metabolites have been found to target specific receptors among the repertoire expressed by the EECs, triggering a hormonal response. It is estimated that in humans almost all fermented SCFA are absorbed by the colonocytes and only 5% are excreted with stool, equivalent to 5–30 millimoles per day. Indeed, it is not practically feasible to measure intraluminal production fluxes of various metabolites in vivo in humans; therefore, most studies focus on the easiest but less informative quantification of fecal SCFA content (393).

Despite the most recent studies of transgenic and germ-free animals, it is still largely unknown by what degree hormones such as GLP-1, and all its related peptides, depend on the microflora, especially in pathologies such as type 2 diabetes.
Recent high-throughput pharmacogenomic studies have deepened our understanding of the molecular players in this human-microbiota relationship. Recently it was shown that a new class of N-acetyl amides is produced by the microbiota, and target GPCRs expressed by the enteroendocrine cells, modulating GLP-1 expression and overall glucose metabolism. In particular, N-oleoyl serinol (N-OS) is described as a potent GPR119 agonist, acting in the lower micromolar range with twice the efficacy of the endogenous ligand OEA (394).

From the evolutionary perspective, dietary components, together with the microbiota-fermented products, have activated the enteroendocrine system for billions of years, since the dawn of metazoan. Considering the vast and continuous pool of metabolites produced and modulated by the microbiota, the distinction between orthosteric and allosteric ligand becomes blurred; different molecules are likely working in synergy to elicit a specific hormonal response.

Modulation of the microbiome has shown promising results in the treatment of type 2 diabetes. For example, recently it was reported that a rhubarb extract, Rhein, increasing the intestinal population of Bacteroidetes, mediates an increase in ileal GLP-1 producing cells, peripheral GLP-1(1-7-36)NH₂ levels and improved glucose tolerance in diabetic db/db mice (395). Consistently, STZ-treated rats, are protected from oxidative and inflammatory stress when treated with Liraglutide, and Bacteroides, as well as Lactobacilli strain populations appear to be restored (396).

In the last decade, the scientific community has just started to unveil the molecular pathways produced by this long-lasting symbiosis. It appears that SCFAs not only induce the release of GLP-1, they also represent a mitogenic signal. Rats fed oligofructose, a substrate for the colonic microbiota which leads to higher SCFAs levels, possess an increased number of colonic L-cells (397). This has been confirmed ex-vivo in human and mouse small intestinal crypts organoids (398).

Other compounds such as bile salts and xenobiotics (399), are known to be metabolized and excreted by the microbiota, affecting the host physiology. Indeed, the pharmacokinetic and pharmacodynamics of any drug taken by mouth should be appraised considering the role of the microbiota, as the varied efficacy of some chemotherapeutics such as 5-FU has been proven to directly depend on this host-microbiota metabolism (400). Even though the anatomical intestinal rearrangement of RYGB and SG patients is known to affect the microbiota, this doesn’t appear to result in a different bile acid metabolism in a rat model (401).

We are at the beginning of a new branch of medical practice, tailored not only to the single person genome, but also to the microbiome.

Future human studies will help us to better understand the big picture of this relationship, to hopefully provide mechanistic knowledge upon which new treatments could be created, such as microbiome-directed gene-therapies for the management of metabolic diseases.

**CONCLUSION AND PERSPECTIVE**

GLP-1R-independent signaling of GLP-1, its intra-islet axis, and its once-thought inactive metabolites, all represent new important additions to our understanding of this peptide in health and disease.

Omnivores’ gastrointestinal tract has co-evolved in strict relationship with a dynamic microbiota and a complex seasonal and regional diet, resulting into a robust and flexible system tightly interconnected via multiple neuroendocrine axes with different organs.

In nature, dietary fats are scarce energy-dense nutrients primarily found in fish and meat. This evolutionary pressure over millions of years has shaped a system for the attentive sensation, assimilation and storage of precious bioactive molecules in all superior animals.

Sensation happens at multiple levels with a plethora of somewhat redundant intestinal receptors (402), specifically in the enteroendocrine cell system. This redundancy can be seen in transgenic animals, whereby the genetic absence of a single chemosensor doesn’t always result in a phenotype, probably due to metabolic compensation from similar and overlapping pathways.

Virtually all macronutrients are absorbed in the small intestine, where maximal activity of the EECs is ensured, while the colonic and rectal GLP-1 secretion is enforced in response to secondary metabolites even hours after the meal ingestion. This pattern is disrupted in bariatric patients undergoing RYGB surgery, where a remodeled GI tract delivers more nutrients to the large intestine, and changes gut-secretome, including its microbiota.

Attempts to mimic this altered meal processing, such as proximal blockage of nutrient absorption resulting in increased delivery of nutrients in the distal intestine, have shown some promising results in healthy and diabetic humans (403). Although this is more challenging with fats because dietary lipids require partial digestion by lipases to become efficient secretagogues (404, 405). However, distant delivery of free fatty acids, or even Oleoyl-Glycerol and sodium taurocholate have shown negligible effects on peripheral levels of GLP-1 or PYY, satiety and glucose tolerance (311, 406, 407). Similarly, distal delivery of the best known aminoacidic GLP-1-secretagogue, glutamine, has proven ineffective at ameliorating glucose tolerance in both healthy and diabetic subjects (407–409).

Furthermore, a recent report (410) examined the effect of RYGB on lean pigs, and indicates how it is the post-operative GLP-1 (9-36)NH₂ levels that raise, while surprisingly the “active” (7-36)NH₂ peripheral levels were reduced.

Indeed, most authors focus only on the peripheral levels of only one of these two peptide species, vastly excluding GLP-1(28-36) NH₂ and (32-36)NH₂ activity, rendering the overall understanding of each individual GLP-1 species, in both health and disease, difficult to discern.

Technical advances ELISA, capable to specifically dissect these peptide species locally and peripherally, will help us to shed new light into this complex physiology (411).

Conclusively, bearing in mind that insulinotropic or incretinotropic effects are not secondary to any single receptor modulation, whereby pools of different luminal stimuli act synergistically on tens of different chemosensors during their intestinal transit and absorption, while interacting with the microflora metabolism, rendering the restoration of a healthy
physiology in diabetic patients with the pharmacological correction of a single axis, highly improbable.

The final dissection of the molecular axis causative of either metabolic syndrome will need more evidence regarding the localized and inter-neuronal physiology of GLP-1 in physiological and pathological statuses. To ultimately tease apart any possible cause from secondary events, species-specific biology will also need to be carefully dissected and interpreted.

AUTHOR CONTRIBUTIONS

SP researched and interpreted all the data from available scientific literature on the PUBMED database, organized, wrote and revised the whole manuscript. SP also conceptualized and drew all the figures assembling the final formatted review. MF conceived, organized, wrote and revised the whole manuscript.

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REFERENCES

1. Roth K, Kim S, Gordon J. Immunocytochemical studies suggest two pathways for enteroenocrine cell differentiation in the colon. *Am J Physiol* (1992) 263(2 Pt 1):G174–G180
2. Griibble FM, Reimann F. Enteroenocrine cells: chemosensors in the intestinal epithelium. *Annu Rev Physiol* (2016) 78:277–99. doi: 10.1146/annurev-physiol-021115-105439
3. Hansen CE, Vrang N, Sangild PT, Jelsing J. Novel insight into the distribution of L-cells in the rat intestinal tract. *Am J Physiol Gastrointest Liver Physiol* (2013) 3:347–358.
4. Habib A, Richards P, Rogers G, Reimann F, Griibble F. Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells. *Clin Exp Diabetes Metabol* (2013) 56:1413–6. doi:10.1007/s00123-013-2887-2
5. Canelas J, Prieto PG, Villanueva-Peñacarrillo ML, Valverde I, Malaise W. Effects of an olive oil-enriched diet on glucagon-like peptide 1 release and intestinal content, plasma insulin concentration, glucose tolerance and cardiovascular safety and pancreatic insulin content in an animal model of type 2 diabetes. *Horm Metab Res* (2006) 38:98–105. doi: 10.1055/s-2006-925126
6. Mace OJ, Tehan B, Marshall F. Pharmacology and physiology of gastrointestinal enteroneocrine cells. *Pharmacol Res Perspect* (2015) 3:e00155. doi: 10.1002/prp2.155
7. Piommelli D. A fatty gut feeling. *Trends Endocrinol Metabol* (2013) 24:332–41. doi:10.1016/j.tem.2013.03.001
8. Drucker D. Glucagon-like peptides: regulators of cell proliferation, differentiation, and apoptosis. *Mol Endocrinol* (2003) 17: 161–71. doi: 10.1210/me.2002-0306
9. Moran GW, Leslie FC, Levison SE, Worthington J, McLaughlin JT. Enteroendocrine cells: neglected players in gastrointestinal disorders? *Mol Endocrinol* (2003) 17: 161–71. doi:10.1210/me.2002-0306
10. Cancelas J, Prieto PG, Villanueva-Peñacarrillo ML, Valverde I, Malaise W. Effects of an olive oil-enriched diet on glucagon-like peptide 1 release and intestinal content, plasma insulin concentration, glucose tolerance and cardiovascular safety and pancreatic insulin content in an animal model of type 2 diabetes. *Horm Metab Res* (2006) 38:98–105. doi: 10.1055/s-2006-925126
11. Moore B. On the treatment of Diabetus mellitus by acid extract of Duodenal Mucous Membrane. *Biochem J* (1906) 1:28.
12. La Barre J. Sur les possiblités d’un traitement du diabète par l’incrétine. *Bull Acad R Med Belg.* (1932) 12:620–34.
13. Perley MJ, Kipnis DM. Plasma insulin responses to oral and intravenous glucose: studies in normal and diabetic subjects. *J Clin Invest* (1967) 46:1954–1962.
14. Drucker DJ. The biology of incretin hormones. *Cell Metabol* (2006) 3:153–65. doi:10.1016/j.cmet.2006.01.004
15. Vilbgbell T, Krappe T, Deacon CF, Madsbad S, Holst JJ. Reduced postprandial concentrations of intact biologically active glucagon-like peptide 1 in type 2 diabetic patients. *Diabetes* (2001) 50:609–13. doi: 10.2337/diabetes.50.3.609
16. Manell H, Staaf J, Manukyan L, Kristinsson H, Cen J, Stenlid R, et al. Altered plasma levels of glucagon, GLP-1 and glicentin during OGTT in adolescents with obesity and Type 2 diabetes. *J Clin Endocrinol Metabol.* (2016) 101:1118–9. doi: 10.1210/jc.2015-3885
17. Wewer Albrechtsen NJ, Hornburg D, Albrechtsen R, Svendsen B, Torang S, Jepsen SL, et al. Oxymodulin identified as a marker of type 2 diabetes and gastric bypass surgery by mass-spectrometry based profiling of human plasma. *EBioMed.* (2016) 7:112–20. doi: 10.1016/j.ebiom.2016.03.034
18. Meier J, Hucking K, Holst J, Deacon C. Reduced insulinoergic effect of gastric inhibitory polypeptide in first-degree relatives of patients with type 2 diabetes. *Diabetes* (2001) 50:2497–2504. doi: 10.2373/diabetes.50.11.2497
19. Nauck MA, Meier J. Incretin hormones: Their role in health and disease. *Diabetes Obesity Metabol.* (2018) 20(Suppl 1):5–21. doi: 10.1111/dom.13129
20. Holst JJ, Pedersen J, Wewer Albrechtsen NJ, Knop FK. The Gut: a key to the pathogenesis of type 2 diabetes? *Metabol Syndr Relat Disord.* (2017) 15:259–62. doi: 10.1089/met.2017.0015
21. Gil-Lozano M, Mingomataj EL, Wu WK, Ridout SA, Brubaker PL. Circadian secretion of the intestinal hormone GLP-1 by the rodent L cell. *Diabetes* (2014) 63:3674–85. doi: 10.2373/diabetes.14-15101
22. Martchenko A, Oh RH, Wheeler SE, Gurges P, Chalmers JA, Brubaker PL. Suppression of circadian secretion of glucagon-like peptide-1 by the saturated fatty acid, palmitate. *Acta Physiol (Oxford, England)* (2018) 222:e13007. doi: 10.1111/apha.13007
23. Nanniopier M, Baldi S, Mari A, Colligiani D, Guarino D, Camastra S, et al. Roux-en-Y gastric bypass and sleeve gastrectomy: mechanisms of diabetes remission and role of gut hormones. *J Clin Endocrinol Metabol.* (2013) 98:4391–9. doi: 10.1210/jc.2013-2538
24. Choi YY, Noh SH, An KY. A randomized controlled trial of Roux-en-Y gastrojejunostomy vs. gastroduodenostomy with respect to the improvement of type 2 diabetes mellitus after distal gastrectomy in gastric cancer patients. *PLoS ONE* (2017) 12:e0188904. doi: 10.1371/journal.pone.0188904
25. Dalsgaard N, Brønden A, Vilbøll T, Knop F. Cardiovascular safety and benefits of GLP-1 receptor agonists. *Expert Opin Drug Saf.* (2017) 16:351–63. doi: 10.1080/14740338.2017.1281246
26. Bajaj HS, Al-Jahri B, Verma S. Glucagon-like peptide-1 receptor agonists and cardiovascular protection in type 2 diabetes: a pathophysiology-based review of clinical implications. *Curr Opin Cardiol.* (2018), doi: 10.1097/hco.0000000000000562. [Epub ahead of print].
27. Bildtner A, Kopf S, Zeier M, Scheurlen KFL, Schulte TM, Kemnott HG, et al. Renal function in type 2 diabetes following gastric bypass. *Dtsch Arztebl Int.* (2016) 113:827–833. doi: 10.3238/arztebl.2016.0827
28. Dieter BP, Alicic RZ, Tuttle KR. GLP-1 Receptor agonists in diabetic kidney disease: from the Patient-Side to the Bench-Side. *Am J Physiol Renal Physiol.* (2018). doi: 10.1152/ajprenal.00211.2018. [Epub ahead of print].
29. Ten Kulve JS, Veltman DJ, Gerdes VEA, van Bloemendaal L, Barkhof F, Deacon CF, et al. RG IJ elevated postoperative endogenous GLP-1 levels mediate effects of roux-en-Y Gastric bypass on neural responsibility to food cues. *Diabetes care* (2018) 40:1522–1529. doi: 10.2337/dc16-2113
30. Jirapinyo P, Jin DX, Qazi T, Mishra N, Thompson CC. A meta-analysis of GLP-1 after roux-en-y gastric bypass: impact of surgical technique and measurement strategy. *Obesity Surg.* (2018) 28:615–26. doi: 10.1007/s11695-017-2913-1

31. Mokadem M, Zechner JF, Margolskee RF, Drucker DJ, Aguirre V. Effects of Roux-en-Y gastric bypass on energy and glucose homeostasis are preserved in two mouse models of functional glucagon-like peptide-1 deficiency. *Mol Metabol.* (2014) 3:191–201. doi: 10.1016/j.molmet.2013.11.010

32. Vetter ML, Wadden TA, Teff KL, Khan ZF, Carvajal R, Ritter S, et al. GLP-1 plays a limited role in improved glycemia shortly after Roux-en-Y gastric bypass: a comparison with intensive lifestyle modification. *Diabetes* (2015) 64:434–46. doi: 10.2337/db14-0558

33. Wilson-Perez HE, Chambers AP, Ryan KK, Li B, Sandoval DA, Stoffers D, et al. Vertical sleeve gastrectomy is effective in two genetic mouse models of glucagon-like Peptide-1 receptor deficiency. *Diabetes* (2013) 62:2386–9. doi: 10.2337/db12-1498

34. Ye J, Hao Z, Mumphrey MR, Townsend RL, Patterson LM, Stylopoulos N, et al. GLP-1 receptor signaling is not required for reduced body weight after RYGB in rodents. *Am J Physiol Regul Integr Comp Physiol.* (2014) 306:R352–362. doi: 10.1152/ajpregu.00491.2013

35. Guida C, Stephen S, Guittion R, Ramacheya RD. The Role of PYY in pancreatic islet physiology and surgical control of diabetes. *Trends Endocrinol Metab.* (2017) 28:262–36. doi: 10.1016/j.tem.2017.04.005

36. Garibay D, McGavigan AK, Lee SA, Fox AL, Michael MD, et al. beta-cell glucagon-like peptide-1 receptor contributes to improved glucose tolerance after vertical sleeve gastrectomy. *Endocrinology* (2016) 157:3405–9. doi: 10.1210/en.2016-1302

37. Albaugh VL, Banan B, Ajouz H, Abumrad NN, Flynn CR. Bile acids and bariatric surgery. *Mol Aspects Med.* (2017) 56:75–89. doi: 10.1016/j.mam.2017.04.001

38. Patti ME, Houten SM, Bianco AC, Bernier R, Larsen PR, Holst JJ, et al. Serum bile acids are higher in humans with prior gastric bypass: potential contribution to improved glucose and lipid metabolism. *Obesity (Silver Spring, Md.)* (2009) 17:1671–7. doi: 10.1038/oby.2009.102

39. Moreno-Arciniegas A, Falchenheiner-Soria J, Bancalero-de Los Reyes J, Camacho-Ramirez A, de Los Angeles Mayo-Ossorio M, Pacheco-Garcia JM, et al. The main participation of the enteroendocrine GLP-1 after bariatric surgery. *Minerva Chir.* (2018). doi: 10.23736/s0002-4733.18.07681-2. [Epub ahead of print].

40. Meier JJ, Nauck MA. Incretin-based therapies: where will we be 50 years from now? *Diabetologia* (2015) 58:1745–50. doi: 10.1007/s00125-015-3608-6

41. Creutzfeldt W. Origin, chemistry, physiology, and pathophysiology of the gastrointestinal hormones. In: *International Symposium. Wiesbaden: Schattauer* (1970).

42. Eisele R, Goke R, Willemer S, Harthus HP, Vermeer H, Arnold R, et al. The main participation of the enterohormone GLP-1 after bariatric surgery. *Cell Tissue Res.* (2014) 357:63–9. doi: 10.1007/s00441-014-1886-9

43. Spreckley E, Murphy KG. The L-cell in nutritional sensing and the regulation of GLP-1 amidation efficiency along the length of the intestine in mice, rats and pigs and in GLP-1 secreting cell lines. *Peptides* (2014) 55:52–7. doi: 10.1016/j.peptides.2014.01.020

44. Thanasupawat T, Hammje K, Adham I, Ghia JE, Del Bigio MR, Krc R, et al. ENSLs activates multiple signalling pathways and regulates GLP-1 secretion in NCI-H716 cells. *J Mol Endocrinol.* (2018) 60:213–24. doi: 10.1530/jme-17-0152

45. Verhoeckx K, Cotter P, Lopez-Expósito I, Kleveiland C, Lea T, Mackie A, et al. The Impact of Food Incretives on Health: In vitro and Ex Vivo Models. *Springer International Publishing* (2016). doi: 10.1007/978-3-19-16104-4

46. Kühre RE, Wewer Albrechtsen NJ, Deacon CF, Balk-Moller E, Rehfeld JF, Reimann F, et al. Peptide production and secretion in CELTag, NCI-H716, and STC-1 cells: a comparison to native L-cells. *J Mol Endocrinol.* (2016) 56:201–11. doi: 10.1530/jme-15-0293

47. Grosse J, Heffron H, Burling K, Hassoun MA, Habib AM, Rogers GI, et al. Insulin-like peptide 5 is an orxigastic gastrointestinal hormone. *Proc Natl Acad Sci. USA.* (2014) 111:11133–8. doi: 10.1073/pnas.1411411111

48. Moriya R, Shirakura T, Ito J, Mashiko S, Seo T. Activation of sodium-glucose cotransporter 1 ameliorates hyperglycemia by mediating incretin secretion in mice. *Am J Physiol Endocrinol Metab.* (2009) 297:E1358–65. doi: 10.1152/ajpendo.00412.2009

49. Bohórquez DV, Shahid RA, Erdmann A, Kreger AM, Wang Y, Calakos N, et al. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest.* (2015) 125:782. doi: 10.1172/JCI78361

50. Anini Y, Fu-Cheng X, Cuber JC, Kervran A, Chariot J, Roz C. Comparison of the postprandial release of peptide YY and proglucagon-derived peptides in the rat. *Pflugers Arch.* (1999) 438:299–306.

51. Tuduri E, Betria D, Porteiro B, Lopez M, Dieguez C, Nogueiras R. Acute but not chronic activation of brain glucagon-like peptide-1 receptors enhances glucose-stimulated insulin secretion in mice. *Diabetes Obesity Metabol.* (2017) 15:789–79. doi: 10.1111/dom.12488

52. Aitwood MM, Krishnan A, Almen MS, Schioth HB. Highly diversified expansions shaped the evolution of membrane bound proteins in metazoans. *Science* (2017) 1:12387. doi: 10.1126/science.aam8154

53. Kenakin T. New lives for seven transmembrane receptors as drug targets. *Trends Pharmacol Sci.* (2015) 36:705–6. doi: 10.1016/j.tips.2015.09.004

54. Milligan G, Shimpukade B, Ulven T, Hudson BD. Complex pharmacology of free fatty acid receptors. *Chem Rev.* (2017) 117:67–110. doi: 10.1021/acs.chemrev.6b00356

55. Kessenbrock M, Groth G. Circular dichroism and fluorescence spectroscopy to study protein structure and protein-protein interactions in ethylene signaling. *Methods Mol Biol.* (2017) 157:341–59. doi: 10.1007/978-1-4939-6854-1_12

56. Safdari HA, Pandey S, Shukla AK, Dutta SK, Dutta SK. Illuminating GPCR Signaling by Cryo-EM. *Sci Rep.* (2018) 8:532–9. doi: 10.1016/j.cmet.2008.11.002

57. Taubch, Cherezov V. Serial femtosecond crystallography of G protein-coupled receptors. *Annu Rev Biophys.* (2018) 47:377–97. doi: 10.1146/annurev-biophys-070317-033229

58. Kahsai AW, Pani B, Leitkowitz RJ. GPCR signaling: conformational activation of arrestins. *Cell Res.* (2018) 28:783–4. doi: 10.1042/ncr20180067-x

59. de Graaf C, Donnelly D, Wootten D, Lau J, Sexton P, Miller L, et al. Glucagon-like peptide-1 and its class B G protein-coupled receptors: a long march to therapeutic successes. *Pharmacol Rev.* (2016) 68:954–1013. doi: 10.1124/pr.115.111395
70. Zhang X, Cai C, Winters M, Wells M, Wall M, Lanter J, et al. Design, synthesis and SAR of a novel series of heterocyclic phenylpropanoic acids as GPR120 agonists. Bioorganic Med. Chem. Lett. (2017) 27:3272–8. doi: 10.1016/j.bmcl.2017.06.028

71. Husted AS, Traulsen M, Rudenko O, Hjortj SA, Schwartz TW. GPCR-mediated remodeling of metabolites. Cell Metab. (2017) 25:777–96. doi: 10.1016/j.cmet.2017.03.008

72. Sun EW, de Fontgalland D, Rabbitt P, Hollington P, Sposato L, Due SL, et al. Mechanisms controlling glucose-induced GIP-1 secretion in human small intestine. Diabetes (2017) 66:2144–9. doi: 10.2337/db17-0058

73. Ding X, Hu CA, Huang P, Li Y, He S, Yang H, et al. Expression of sweet taste receptor and gut hormone secretion in modelled type 2 diabetes. General Comp Endocrinol. (2017) 252:142–9. doi: 10.1016/j.ygcen.2017.08.008

74. Lindqvist A, Shcherbina L, Fischer AT, Wierup N. Ghrelin is a regulator of glucagon-like peptide-1 secretion and transcription in mice. Front Endocrinol. (2017) 8:135. doi: 10.3389/fendo.2017.00135

75. Feng R, Qian C, Liu Q, Jin Y, Liu L, Li S, et al. Expression of sweet taste receptor and gut hormone secretion in modelled type 2 diabetes. General Comp Endocrinol. (2017) 252:142–9. doi: 10.1016/j.ygcen.2017.08.008

76. Reimann F, Gribble F. G protein-coupled receptors as new therapeutic targets for type 2 diabetes. Clin Exp Metab. (2016) 59:229–33. doi: 10.1007/s00125-015-3825-z

77. Lin HV, Wang J, Wang J, Li W, Wang X, Alston JT, et al. GPR142 prompts ingestion. Mol Metab. (2018) 37:4657. doi: 10.1016/j.molmet.2018.07.030

78. Lakshmi K, Goto H, Miura H, Suzuki S, et al. GPR120 as a regulator of the upper small intestine and has a critical role in GIP secretion after fat ingestion. J Clin Endocrinol Metab. (2017) 102:827. doi: 10.1210/jc.2017-00275

79. Oya M, Kitaguchi T, Pais R, Reimann F, Gribble F, Tsuibo T. The G protein-coupled receptor family C group 6 subtype A (GPR6CA) receptor is involved in amino acid-induced glucagon-like peptide-1 secretion from GI/UT cells. J Biol Chem. (2013) 288:4513–21. doi: 10.1074/jbc.M112.402677

80. Gupta V. (2012). Pleiotropic effects of incretins. J Biol Chem. (2013) 288:4513–21. doi: 10.1074/jbc.M112.402677

81. Paternoster and Falasca Regulation of GLP-1 Secretion

82. Tough IR, Forbes S, Herzog H, Jones RM, Schwartz TW, Cox HM. Bidirectional GPR119 agonism requires peptide YY and glucagon for activity in mouse and human colon mucosa. Endocrinology (2018) 159:704–17. doi: 10.1210/en.2017-03172

83. Patel S, Mace Of, Tough IR, White J, Cock TA, Warrman Berglund U, et al. Gastrointestinal hormonal responses on GPR119 activation in lean and diseased rodent models of type 2 diabetes. Int J Obes. (2014) 38:1365. doi: 10.1038/ijo.2014.10

84. Li NX, Brown S, Kowalski T, Wu M, Yang L, Dai G, et al. GPR119 Agonism increases glucagon secretion during insulin-induced hypoglycemia. Diabetes (2018) 67:1401–13. doi: 10.2337/db18-0031

85. Yamada Y, Terachi Y, Watada H, Nakatsu Y, Shiosakai K, Washio T, et al. Efficacy and Safety of GPR119 Agonist DS-8500a in Japanese Patients with Type 2 Diabetes: a Randomized, Double-Blind, Placebo-Controlled, 12-Week Study. Adv Ther. (2018) 35:367–81. doi: 10.1007/s12325-018-0668-2

86. Christensen MB. Glucose-dependent insulinotropic polypeptide: effects on insulin and glucagon secretion in humans. Dan Med J. (2016) 63:B5230. Available online at: https://pdfs.semanticscholar.org/b19d/0b5bd402a2929e2c6fb01d6cdd7eaa767e.pdf

87. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab. (1973) 37:826–8. doi: 10.1207/jcem-37-5-826

88. Piro S, Mascali LG, Urbano F, Filippello A, Malaguarnera R, Calanna S, et al. Chronic exposure to GLP-1 increases GLP-1 synthesis and release in a pancreatic alpha cell line (alpha-TC1): evidence of a direct effect of GLP-1 on pancreatic alpha cells. PlcOs ONE (2014) 9:e90093. doi: 10.1371/journal.pone.0090093

89. Sankoda A, Tsukasa Y, Arai T, Yamada M, et al. Free fatty acids regulate gut incretin glucagon-like peptide-1 secretion through Endosomal co-localization of GLP-1/GLP-1R complex functioning through intra-islet paracrine mechanism. Sci Rep. (2018) 8:3725. doi: 10.1038/s41598-018-21751-w

90. Kumar DP, Asgharpour A, Mirshahi F, Park SH, Liu S, Imai Y, et al. Activation of transmembrane bile acid receptor TGR5 modulates pancreatic islet alpha cells to promote glucose homeostasis. J Biol Chem. (2016) 291:26626–40. doi: 10.1074/jbc.M116.799504

91. Bradfield CA, Sghar R, Ruckel A, Roy S, Meehan B, Turner S, et al. Nucleic Acids Res. (1996) 24:4719–4730

92. Holst Jj, Bensani M, Johnsen AH, Kofod H, Hartmann B, Orskov C. Proglucagon processing in porcine and human pancreas. J Biol Chem. (1994) 269:18827–18833.
active GLP-1 levels in non-diabetic humans. *Obesity Surg.* (2014) 24:241–52. doi: 10.1007/s11695-013-1066-0

147. Salehi M, Prignon RL, D’Alessio DA. Gastric bypass surgery enhances glucagon-like peptide 1-stimulated postprandial insulin secretion in humans. *Diabetes* (2017) 66:2308–14. doi: 10.2337/db17-0230

148. Davis DB, Khoraki J, Ziemelis M, Sirinvaravong S, Han JY, Campos GM. GLP-1 Receptor system. *Diabetes* (2018) 9:15–27. doi: 10.2337/db17-0270.2017.12.01

149. Drucker DJ, Nauck MA. The incretin system: glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors in type 2 diabetes. *Lancet* (2006) 368:1696–705. doi: 10.1016/S0140-6736(06)69705-5

150. Meier J, Nauck M. Incretins and the development of type 2 diabetes. *Curr Diab Rep.* (2006) 6:194–201.

151. Nauck MA, Stöckmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in Type 2 (non-insulin-dependent) diabetes. *Clin Exp Diabet Metab.* (1986) 29:46–52. doi: 10.1007/BF00427280

152. Holst J. (2006). Glucagon-like peptide-1: from extract to agent. The Claude Bernard Lecture, 2005. *Clin Exp Diab Metab.* 49:253–260. doi: 10.1007/s11695-005-0107-0

153. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 7-36 amide but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. *J Clin Invest.* (1993) 93:301–6. doi: 10.1172/JCI116186

154. Guida C, McCulloch LJ, Godzagar M, Stephen SD, Baker C, Basco D, et al. Staglgin and Roux-en-Y gastric bypass modulate insulin secretion via regulation of intra-islet PYY. *Diabetes Obesity Metabol.* (2018) 20:571–81. doi: 10.1111/dom.13113

155. Lefort S, Tschop MH, Garcia-Caceres C. A synaptic basis for GLP-1 action in the brain. *Neuron* (2017) 96:713–5. doi: 10.1016/j.neuron.2017.10.034

156. Liu J, Conde K, Zhang P, Lilascharoen V, Xu Z, Lim BK, et al. Enhanced AMPA receptor trafficking mediates the anorexigenic effect of endogenous glucagon-like peptide-1 in the paraventricular hypothalamus. *Neuron* (2017) 96:897–909.e895. doi: 10.1016/j.neuron.2017.09.042

157. Vrang N, Larsen PJ, Tang-Christensen M, Jessop DS. Central administration of glucagon-like peptide-1 (GLP-1) is an anorectic signal in healthy humans. *Diabetes* (2016) 65:1714–23. doi: 10.2337/db15-1141

158. Pyke C, Heller RS, Kirk RK, Orskov C, Reitz-Durante S, Kaastrup P, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* (2014) 155:1280–90. doi: 10.1210/en.2013-1934

159. Boirade E, Bloch O, Ben-Yehudah G, Cantrell D, Shirin H, Rapoport MJ. GLP-1 receptor is expressed in human stomach mucosa: analysis of its cellular association and distribution within gastric glands. *J Histochem Cytochem.* (2013) 61:649–58. doi: 10.1369/0022155413497586

160. Wissmann P, Barkholt P, Secher T, Vrang N, Hansen HB, Jeppesen PB, et al. The endogenous proglucagon system is not essential for gut growth homeostasis in mice. *Mol Med.* (2017) 6:681–92. doi: 10.1016/j.molmed.2017.04.007

161. List JF, He H, Habener JE. Glucagon-like peptide-1 receptor and proglucagon expression in mouse skin. *Regul Peptiides* (2006) 134:149–57. doi: 10.1016/j.regpep.2006.02.007

162. Cameron-Vendrig A, Reheman A, Siraj MA, Xu XR, Wang Y, Lei X, et al. GLP-1 receptor expression attenuates platelet aggregation and thrombosis. *Diabetes* (2016) 65:1714–23. doi: 10.2337/db15-1141

163. Pyke C, Hoffer RS, Kirk RK, Orskov C, Reitz-Durante S, Kaastrup P, et al. GLP-1 receptor localization in monkey and human tissue: novel distribution revealed with extensively validated monoclonal antibody. *Endocrinology* (2014) 155:1280–90. doi: 10.1210/en.2013-1934

164. Ayala JE, Bracy DP, James FD, Burmeister MA, Wasserman DH, Drucker DJ. Glucagon-like peptide-1 receptor knockout mice are protected from high-fat diet-induced insulin resistance. *Endocrinology* (2010) 151:4678–87. doi: 10.1210/en.2010-0289

165. Knauf C, Cani PD, Ait-Belgnaoui A, Benani A, Dray C, Cabou C, et al. Brain glucagon-like peptide-1 signaling controls the onset of high-fat diet-induced insulin resistance and reduces energy expenditure. *Endocrinology* (2008) 149:4768–77. doi: 10.1210/en.2008-0180

166. Cheang JY, Moyle PM. Glucagon-like peptide-1-based therapeutics: current status and future opportunities beyond type 2 diabetes. *ChemMed Chem.* (2013) 6:662–71. doi: 10.1002/cmdc.201700781

167. Defronzo R. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* (2009) 58:773–95. doi: 10.2337/db08-0928

168. Demir S, Temizkran S, Sargin M. C-peptide levels predict the effectiveness of dipeptidyl peptidase-4 inhibitor therapy. *J Diabetes Res.* (2016) 2016:459603. doi: 10.1155/2016/459603

169. Boussagen R, Bejan-Angoultant V, Saadatian-Eliahi M, Lafont S, Bergeanneau C, Kassai C, et al. Brain glucagon-like peptide-1 signaling controls the onset of high-fat diet-induced insulin resistance and reduces energy expenditure. *Endocrinology* (2008) 149:4768–77. doi: 10.1210/en.2008-0180

170. Cheang JY, Moyle PM. Glucagon-like peptide-1 (GLP-1)-based therapeutics: current status and future opportunities beyond type 2 diabetes. *ChemMed Chem.* (2013) 6:662–71. doi: 10.1002/cmdc.201700781

171. Defronzo R. From the triumvirate to the ominous octet: a new paradigm for the treatment of type 2 diabetes mellitus. *Diabetes* (2009) 58:773–95. doi: 10.2337/db08-0928

172. Demir S, Temizkran S, Sargin M. C-peptide levels predict the effectiveness of dipeptidyl peptidase-4 inhibitor therapy. *J Diabetes Res.* (2016) 2016:459603. doi: 10.1155/2016/459603

173. Behme MT, Dupré J, McDonald TJ. Glucagon-like peptide 1 improved glycemic control in type 1 diabetes. *BMC Endocrine Disorders* (2003) 3:3. doi: 10.1186/1472-6833-3-3

174. Frandsen CS, Dejgaard TF, Madshad S, Holst JJ. Non-insulin pharmacological therapies for treating type 1 diabetes. *Expert Opin Pharmacother.* (2018) 19:947–60. doi: 10.1080/14656566.2018.1483339
185. Hawkes N. Sixty seconds on ... semaglutide. BMJ (2017) 359:j5010. doi: 10.1136/bmj.j5010

186. Bond A. Exenatide (Byetta) as a novel treatment option for type 2 diabetes mellitus. Proceedings (Baylor University Medical Center) (2006) 19:281–4. doi: 10.1080/08998280.2006.11928181

187. Eng J. Exendin peptides. Mt Sinai J Med. (1992) 59:147–149.

188. Tahrania AA, Bellary S, Barnett AH. Once-weekly GLP-1R agonists: moving the goal posts. Lancet Diabetes Endocrinol. (2018) 6:260–1. doi: 10.1016/s2213-8587(18)30049-4

189. Julia M, Mara KB, Julia O, Robert S, Vera J, Joachim J, et al. Glucagon-Like Peptide-1 and its cleavage products are renoprotective in murine diabetic nephropathy. Diabetes (2018) 67:db171212. doi: 10.2337/db17-1212

190. Ilsen DH, Rolin B, Rakipovski G, Skovsted GF, Madsen A, Kolstrup S, et al. Liraglutide decreases hepatic inflammation and injury in advanced lean non-alcoholic steatohepatitis. Basic Clin Pharmacol Toxicol. (2018). doi: 10.1111/bcpt.13082. [Epub ahead of print]

191. Bae CS, Song J. The role of glucagon-like peptide 1 (GLP1) in type 3 diabetes: Eng J. Exendin peptides. Mt Sinai J Med. (1992) 59:147–149. Exenatide (Byetta) as a novel treatment option for type 2 diabetes. Diabetes (2018) 67:db171212. doi: 10.2337/db17-1212

192. Drucker DJ. The ascending GLP-1 road from clinical safety to pancreatic alpha-cells in mouse models of β-cell regeneration. Islets (2010) 2:149–55. doi: 10.4161/islets.2.3.13199

193. Dhir G, Cusi K. Glucagon like peptide-1 receptor agonists. J Diabetes Invest. (2015) 6:460–6. doi: 10.1111/jdi.12468

194. Khat DZ, Husain M. Molecular mechanisms underlying the cardiovasculat disease: a novel therapeutic option. J Invest Med. (2016) 66:7–10. doi: 10.1111/jim-2017-00554

195. Dror E, Nordmann TM, Goetz N, et al. Unimolecular dual incretins maximize metabolic benefits with type 2 diabetes. Diabetes Obesity Metabol. (2018) 20:1191–6. doi: 10.1111/dom.13703

196. Vatansever and Falasca Regulation of GLP-1 Secretion

197. Bond A. Exenatide (Byetta) as a novel treatment option for type 2 diabetes mellitus. Proceedings (Baylor University Medical Center) (2006) 19:281–4. doi: 10.1080/08998280.2006.11928181

198. Pocai A. Unraveling oxyntomodulin, GLP1's enigmatic brother. Cell Metab. (2016) 24:523–5. doi: 10.1016/j.cmet.2016.04.005

199. Wynne K, Park AJ, Small CJ, Patterson M, Ellis SM, Murphy KG, et al. Pancreatic alpha cell-derived glucagon-related peptides are required for beta cell adaptation and glucose homeostasis. Cell Rep. (2017) 18:3192–203. doi: 10.1016/j.celrep.2017.03.005

200. Smith EP, An Z, Wagner C, Lewis AG, Cohen EB, Li B, et al. The role of beta cell glucagon-like peptide-1 signaling in glucose regulation and response to diabetes drugs. Cell Metab. (2019) 16:1905–10. doi: 10.1016/j.cmet.2014.04.005

201. Anlauf M, Weihe E, Hartschuh W, Hamscher G, Feurle GE. Localization of pancreatic preproglucagon in glucose homeostasis in mice. Cell (1992) 59:147–149.

202. Vasu S, Moffett RC, Thorens B, Flatt PR. Role of endogenous GLP-1 and GIP in beta cell compensatory responses to insulin resistance and cellular stress. PLoS ONE (2014) 9:e101095. doi: 10.1371/journal.pone.0101095

203. Fujita Y, Widerman RD, Asadi A, Yang GK, Baker R, Webber T, et al. Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. Gastroenterology (2010) 138:1966–75. doi: 10.1053/j.gastro.2010.01.049

204. Dalle S, Fontés G, Lajoix AD, LeBrigand L, Gross R, Ribes G, et al. Miniglucagon (glucagon 19–29): a novel regulator of the pancreatic islet physiology. Diabetes (2002) 51:406–12. doi: 10.2337/diabetes.51.2.406

205. Khan D, Vasu S, Moffett RC, Gaut V A, Flatt PR, Irwin N. Locally produced xenin and the neurotensinergic system in pancreatic islet function and beta-cell survival. BioL Chem. (2017) 399:79–92. doi: 10.1515/bch-2017-0136

206. Luo X, Li T, Zhu Y, Dai Y, Zhao J, Guo ZY, et al. The insulinotropic effect of the insulinotrophic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. Gastroenterology (2010) 138:1966–75. doi: 10.1053/j.gastro.2010.01.049

207. Vasu S, Moffett RC, Thorens B, Flatt PR. Role of endogenous GLP-1 and GIP in beta cell compensatory responses to insulin resistance and cellular stress. PLoS ONE (2014) 9:e101095. doi: 10.1371/journal.pone.0101095

208. Fujita Y, Widerman RD, Asadi A, Yang GK, Baker R, Webber T, et al. Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion. Gastroenterology (2010) 138:1966–75. doi: 10.1053/j.gastro.2010.01.049

209. Dalle S, Fontés G, Lajoix AD, LeBrigand L, Gross R, Ribes G, et al. Miniglucagon (glucagon 19–29): a novel regulator of the pancreatic islet physiology. Diabetes (2002) 51:406–12. doi: 10.2337/diabetes.51.2.406

210. Khan D, Vasu S, Moffett RC, Gaut V A, Flatt PR, Irwin N. Locally produced xenin and the neurotensinergic system in pancreatic islet function and beta-cell survival. BioL Chem. (2017) 399:79–92. doi: 10.1515/bch-2017-0136

211. Liu P, Song J, Liu Y, Fan H, Te W, Wang L, et al. Insulin regulates glucagon-like peptide-1 secretion by pancreatic alpha cells. Endocrine (2018). doi: 10.1007/s12020-018-1684-3. [Epub ahead of print]
246. Ohtsu Y, Burcelin R. GLP-1 effects on islets: hormonal, neuronal, or paracrine? *Diabetes care* (2013) 36 (Suppl 2):S145–148. doi: 10.2337/dcs13-2015

247. Wueste S, Laessner CI, Boni-Schnetzler M, Item F, Lucchini FC, Borsigova M, et al. IL-6-Type cytokine signaling in adipocytes induces intestinal GLP-1 secretion. *Diabetes* (2018) 67:36–45. doi: 10.2337/db17-0637

248. Lang Lehnikov L, Lynghaek MP, Soederlund L, Legaard GE, Elshe JA, Heywood SE, et al. Interleukin-6 delays gastric emptying in humans with direct effects on glycemic control. *Cell Metab.* (2018) 27:1201–11.e1203. doi: 10.1016/j.cmet.2018.04.008

249. Lebrun LJ, Lenaerts K, Kiers D, Pias de Barros JP, Le Guern N, Ple snik J, et al. Enteroendocrine L cells sense LPS after gut barrier injury to enhance GLP-1 secretion. *Cell Rep.* (2017) 21:1168–80. doi: 10.1016/j.celrep.2017.10.008

250. Chen T, Tian P, Huang Z, Zhao X, Wang H, Xia C, et al. Engineered commensal bacteria prevent systemic inflammation-induced memory impairment and amyloidogenesis via producing GLP-1. *Appl Microbiol Biotechnol.* (2018) 102:7565–75. doi: 10.1007/s00253-018-9155-6

251. Wierup N, Lehrskov L, Lyngbaek MP, Soederlund L, Legaard GE, Ehses JA, Egerod KL, Engelstoft MS, Dmytriyeva O, Theodorsen E, Lebrun LJ, Lenaerts K, Kiers D, Pais de Barros JP, Le Guern N, Plesnik J, et al. Interleukin-6 delays gastric emptying in humans with direct effects on glycemic control. *Cell Metab.* (2018) 27:1201–11.e1203. doi: 10.1016/j.cmet.2018.04.008

252. Parker HE, Habib AM, Rogers GJ, Gribble FM, Reimann F. Nutrient-dependent secretion of glucose-dependent insulinotropic polypeptide from primary murine K cells. *Diabetologia* (2009) 52:289–98. doi: 10.1007/s00125-008-0812-x

253. Nakanoto K. A new pain regulatory system via the brain long chain fatty acid receptor GPR40/FFA1 signal. *Yakugaku Zasshi* (2013) 137:199–204. doi: 10.1248/yakushi.16-00208

254. Dragano NRV, Solon C, Ramalho AF, de Moura RF, Razolli DS, Christiansen E, et al. Polysaturated fatty acid receptors, GPR40 and GPR120, are expressed in the hypothalamus and control energy homeostasis and inflammation. *J Neuroinflammation* (2017) 14:91. doi: 10.1186/s12974-017-0869-7

255. Kristinsson H, Smith DM, Bergsten P, Sargsyan E. FFAR1 is involved in both the acute and chronic effects of palmitate on insulin secretion. *Endocrinology* (2013) 154:1078–88. doi: 10.1210/en.2013-1352

256. Panse M, Gerst F, Kaiser G, Teutsch CA, Dolker R, Wagner R, et al. Activation of extracellular signal-regulated protein kinases 1 and 2 (ERK1/2) by free fatty acid receptor 1 (FFA1/GPR40) protects from palmitate-induced beta cell death, but plays no role in insulin secretion. *Cell Physiol Biochemi.* (2015) 35:1537–45. doi: 10.1159/0003397969

257. Hojdic E, Olde B, Meidute-Abaraviciene S, Winzell MS, Ahren B, Salehi A. GPR40 is expressed in glucagon producing cells and affects glucagon secretion. *Biochem Biophys Res Comm.* (2007) 354:240–5. doi: 10.1016/j.bbrc.2006.12.193

258. Jackson EK, Dubinion JH, Mi Z. Effects of dipeptidyl peptidase IV inhibition enhances glucagon-like peptide-1 secretion in normal and diabetic rodents. *J Mol. Endocrinol.* (2014) 52:R35–49. doi: 10.1530/jme-13-01122

259. Rostamkhani F, Zardooz H, Goshadrou F, Baveisi M, Hedayati M. Stress induction of FFA1 mediates GLP-1 secretion. *Mol Cell Endocrinol.* (2013) 369:119–129. doi: 10.1016/j.mce.2014.07.004

260. Pachanski MJ, Kirkland ME, Kosinski DT, Mane J, Cheewatrakoolpong B, Pachanski MJ, Mane J, Cheewatrakoolpong B, Latour MG, Alquier T, Oseid E, Tremblay C, Jetton TL, Luo J, et al. GPR40, a C-type lectin receptor expressed on glucagon-producing cells, mediates glucagon-like peptide-1 secretion. *Cell Metab.* (2013) 36 (Suppl 2):S145–148. doi: 10.1016/j.cmet.2018.04.008

261. Gorski JN, Pachanski MJ, Mane J, Plummer CW, Souza S, Thomas-
264. Psichas A, Larraurie PF, Golupsink DA, Gribble FM, Reimann F. Chylomicrons stimulate inciner secretion in mouse and human cells. Diabetesb (2017) 66:2475–85. doi: 10.1007/s00125-017-4420-2

265. Lin DC-H, Guo Q, Luo J, Zhang J, Nguyen K, Chen M, et al. Identification and pharmacological characterization of multiple allosteric binding sites on the free fatty acid 1 receptor. Mol Pharmacol. (2012) 82:843–59. doi: 10.1124/mol.112.079640

266. Little TJ, Isaacs NJ, Young RL, Ott R, Nguyen NQ, Rayner CK, et al. Characterization of duodenal expression and localization of fatty acid-sensing receptors in humans: relationships with body mass index. Am J Physiol Gastroint Liver Physiol. (2014) 307:G598–67. doi: 10.1152/ajpgi.00134.2014

267. Miyatachi S, Hirasea A, Iga T, Liu N, Isubco C, Sadakane K, et al. Distribution and regulation of protein expression of the free fatty acid receptor GPR120. Naunyn Schmiedeberg Arch Pharmacol. (2009) 579:427–34. doi: 10.1007/s00210-008-0390-8

268. van der Wielen N, van Avesaat M, de Wit NJ, Vogels JT, Troost F, Masclee A, et al. Activity of dietary fatty acids on FFA1 and FFA4 and their role in polymorphonuclear cell activation. J Biol Chem. (2018) 293:4935–40. doi: 10.1074/jbc.m209706200

269. Hasan AU, Ohmori K, Hashimoto T, Kimitori K, Yamaguchi F, Noma T, et al. GPR120 in adipocytes has differential roles in the production of pro-inflammatory adipocytokines. Biochem Biophys Res Commun. (2016) 476:86–82. doi: 10.1016/j.bbrc.2016.03.001

270. Schlippeoort M, van Dam AD, Hoege E, Shabalinga IG, Okolo A, Hanyaloglu AC, et al. The GPR120 agonist TUG-891 promotes metabolic health by stimulating mitochondrial respiration in brown fat. EMBO Mol Med. (2018) 10:e8047. doi: 10.15252/emmm.201708047

271. Stone VM, Dhayal S, Brocklehurst KJ, Lenaghan C, Sorhede Winzell M, Hammar M, et al. GPR120 (FFAR4) is preferentially expressed in pancreatic delta cells and regulates somatostatin secretion from murine islets of Langerhans. Diabetologia (2014) 57:1182–91. doi: 10.1007/s00125-014-3213-0

272. Christiansen E, Watterson KR, Stocker CJ, Sokol E, Jenkins L, Simon K, et al. Activity of dietary fatty acids on FAA1 and FAA4 and characterisation of pinolenic acid as a dual FAA1/FFA4 agonist with potential effect against metabolic diseases. Br J Nutr. (2015) 113:1677–88. doi: 10.1017/s000711451500118x

273. Nagasawa T, Nakamichi H, Higashiyama S, Igarashi Y, Mitsuake S. Phytosphingosine is a novel activator of GPR120. J. Biochim. (2018) 164:27–32. doi: 10.1093/jb/mmy017

274. Oh DY, Talukdar S, Bae El, Imamura T, Morinaga H, Fan W, et al. GPR120 is an omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin-sensitizing effects. Cell (2010) 142:687–98. doi: 10.1016/j.cell.2010.07.041

275. Anbazhagan AN, Priyamvada S, Gujral T, Bhattacharyya S, Alrefai WA, Dudeja PK, et al. A novel anti-inflammatory role of GPR120 in intestinal epithelial cells. Am J Physiol Cell Physiol. (2016) 310:C612–21. doi: 10.1152/ajpcell.00123.2015
Ang Z, Xiong D, Wu M, Ding JL. FFAR2-FFAR3 receptor heteromerization regulates GLP-1 secretion. FASEB J. (2018) 32:289–303. doi: 10.1096/fj.201702528R

Forbes S, Stafford JS, Coope G, Heffron H, Real K, Newman R, et al. Selective FFAR2 agonism appears to act via intestinal PYY to reduce transit and food intake but does not improve glucose tolerance in mouse models. Diabetes (2015) 64:3763–71. doi: 10.2373/dbi15-0481

Christiansen CB, Gabe MBN, Svendsen B, Dragsted LO, Rosenkilde MM, Milligan G, Alvarez-Curto E, Hudson BD, Prihandoko R, Tobin AB. Tang C, Ahmed K, Gille A, Lu S, Grone HJ, Tunaru S, et al. Loss of FFA2 and FFA3 increases insulin secretion and improves glucose tolerance in type 2 diabetes. Nat Med. (2015) 21:173–7. doi: 10.1038/nm.3779

Milligan G, Alvarez-Curto E, Hudson BD, Prihandoko R, Tobin AB. FFAR4/GPR120: pharmacology and therapeutic opportunities. Trends Pharmacol Sci. (2017) 38:809–21. doi: 10.1016/j.tips.2017.06.006

Hudson BD, Tikhonova IG, Pandey SK, Ulven T, Milligan G. Extracellular sonic locks determine variation in constitutive activity and ligand potency between species orthologs of the free fatty acid receptors FFA2 and FFA3. J Biol Chem. (2012) 287:41195–209. doi: 10.1074/jbc.M112.396259

Cummings JH, Pomare EW, Branch WJ, Naylor CP, Macfarlane GT. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. Physiol Rev. (2001) 81:1221–77. doi: 10.1152/physrev.2001.81.3.1221

Puhl HL III, Won YJ, Lu VB, Ikeda SR. Human GPR42 is a consensus sequence for GPR119 and GPR42. DNA Cell Biol. (2010) 29:75–87. doi: 10.1177/0739431009353553

Schwartz GJ, Fu I, Astarita G, Li X, Gaetani S, Campolongo P, et al. The lipid messenger OEA links dietary fat intake to satiety. Cell Metab. (2008) 8:281–8. doi: 10.1016/j.cmet.2008.08.005

Astarita G, Rouke BC, Andersen JB, Fu I, Kim JH, Bennett AF, et al. Postprandial increase of oleoylthanolamide mobilization in small intestine of the Burmese python (Python molurus). Am J Physiol Regul Integr Comp Physiol. (2006) 290:R417–22. doi: 10.1152/ajpregu.00664.2005

Tinoco AB, Armriotti A, Isorna E, Delgado MJ, Piomelli D, de Pedro N. Role of oleoylthanolamide as a feeding regulator in goldfish. J Exp Biol (2014) 217 (Pt 15):2761–9. doi: 10.1242/jeb.106161

Diep TA, Madsen AD, Krogh-Hansen S, Al-Shalwani S, Al-Sabagh L, Holst B, et al. Dietary non-esterified oleic acid decreases the jejunal levels of n-acylthanolamines. PLoS ONE (2014) 9:e100365. doi: 10.1371/journal.pone.0100365

Gao J, Tian L, Weng G, O’Brien TD, Luo J, Guo Z. Stimulating beta-cell replication and improving islet graft function by AR231453, a GPR119 agonist. Transplant Proc. (2011) 43:3217–20. doi: 10.1016/j.transproceed.2011.10.021

Moss CE, Glass LI, Diakogiannaki E, Pais R, Lenaghan C, Smith DM, et al. Lipid derivatives activate GPR119 and trigger GLP-1 secretion in primary murine L-cells. Peptides (2016) 77:16–20. doi: 10.1016/j.peptides.2015.06.012

Panaro BL, Flock GB, Campbell JE, Beaudry JL, Gao X, Drucker DJ. beta-Cell Inactivation of Gpr119 Unmasks Incretin Dependence of GPR119-Mediated Glucoregulation. Diabetes (2017) 66:1626–35. doi: 10.2337/db17-0017

Cox HM, Tough IR, Woodson AM, Zhang L, Nguyen AD, Sainsbury A, et al. Peptide YY is critical for acylethanolamine receptor Gpr19-induced activation of gastrointestinal mucosal responses. Cell Metab. (2010) 11:532–42. doi: 10.1016/j.cmet.2010.04.014

Hu YW, Yang YJ, Ma X, Chen ZP, Hu YR, Zhang JY, et al. Expression and distribution of Gpr119 in the pancreatic islets and beta cells: predominant localization in pancreatic polypeptide-secreting PP-cells. Biochem Biophys Res Commun. (2006) 351:475–80. doi: 10.1016/j.bbrc.2006.06.076
Regulation of GLP-1 Secretion

337. Wiley JW, O’Dorisio TM, Owang C. Vasooactive intestinal polypeptide mediates cholecystokinin-induced relaxation of the sphincter of Oddi. J Clin Invest. (1988) 81:1920–4. doi:10.1172/jci13539

338. Katsumi S, Hirasawa A, Tsujimoto G. Bile acids promote glucagon-like peptide-1 secretion through TGR5 in a murine enterodendocrine cell line STC-1. Pancreasm. (2005) 329:386–90. doi:10.1016/j.pancre.2005.01.039

339. Kuhre RE, Wewer Albrechtsen NJ, Larsen O, Jepsen SL, Balk-Moller E, Andersen DB, et al. Bile acids are important and direct regulators of the secretion of appetite- and metabolism-regulating hormones from the gut and pancreas. Mol Metab. (2018) 11:84–95. doi:10.1016/j.molmet.2018.03.007

340. Lefebvre P, Carujo B, Lien F, Kuipers F, Staels B. Role of bile acids and bile acid receptors in metabolic regulation. Physiol Rev. (2009) 89:147–91. doi:10.1152/physrev.00108.2008

341. Bronden A, Alber L, Rohde U, Jabsberg LS, Rehfeld JF, Holst JJ, et al. The bile acid-sequestering resin sevelamer eliminates the acute GLP-1 stimulatory effect of endogenously released bile acids in patients with type 2 diabetes. Diabetes Obes Metab. (2017) 20:362–9. doi:10.1111/dom.13080

342. Adrian TE, Gariballa S, Parekh KA, Thomas SA, Saadi H, Al Kaabi A, et al. The nuclear receptor PXR is a lithocholic acid sensor that mediates the acute effect of lithocholic acid receptor 1 (GPBAR1, TGR5). J Med Chem. (2018) 61:7589–613. doi:10.1021/acs.jmedchem.8b00308

343. Retzci L, Vellani V, Schiano-Moriello A, Marinpi M, Magherini P, Orlando P, et al. Plant-derived cannabinoids modulate the activity of transient receptor potential channels of ankyrin type-1 and melastatin type-8. J Pharmacol Exp Ther. (2008) 325:1007–15. doi:10.1124/jpet.107.134809

344. Caterina MJ, Schumacher MA, Tomagina M, Rosen TA, Levine JD, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature (1997) 398:816–24. doi:10.1038/39807

345. Zhu Z, Luo Z, Ma S, Liu D. TRP channels and their implications in metabolic diseases. Pflag Arch. (2011) 461:211–23. doi:10.1002/pfag.010902-5

346. Liu D, Zhu Z, Tepel M. The role of transient receptor potential channels in metabolic syndrome. Hypertens Res. (2008) 31:1989–95. doi:10.1210/hyp.31.1989

347. Agol SE, Tominaga M, Julius D. Activation of the vanilloid receptor 1 (VR1) by capsaicin is modulated by a key extracellular factor. Proc Natl Acad Sci USA. (2000) 97:8134–9. doi:10.1073/pnas.100129497

348. Bohlen CJ, Priel A, Zhou S, King D, Siemens J, Julius D. A bivalent vanilant tautonl acid activates the capsaicin receptor, TRPV1, by targeting the outer pore domain. Cell (2010) 141:834–45. doi:10.1016/j.cell.2010.03.052

349. Min JW, Liu WH, He XH, Peng BW. Different types of toxins targeting TRPV1 in pain. Toxicon (2013) 71:66–75. doi:10.1016/j.toxicon.2013.05.016

350. Huang HH, Prescott ED, Kong H, Shields S, Jorda SE, Basbaum AI, et al. Bradykinin and nerve growth factor release the capsaicin receptor from PtdIns(4,5)P2-mediated inhibition. Nature (2001) 411:957–62. doi:10.1038/35082088

351. Jia Y, McLeod RW, Wang X, Parra LE, Egan RW, Hey JA. Anandamide induces cough in conscious guinea-pigs through VR1 receptors. Br J Pharmacol. (2002) 137:831–6. doi:10.1038/sj.bjp.0704950

352. Bisogno T, Hanus L, De Petrocellis L, Tchilimon S, Ponde DE, Brandi I, et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. Br J Pharmacol. (2001) 134:845–52. doi:10.1038/sj.bjp.0704327

353. Moriyama T, Higashi T, Togashi K, Iida T, Segi S, Sugimoto Y, et al. Sensitization of TRPV1 by EPI and IP6 reveals peripheral nociceptive mechanism of prostaglandins. Mol Pain (2005) 1:3. doi:10.1186/1744-8009-1-3

354. Amadesi S, Nieu P, Largentte N, Cottrell GS, Grady EF, Trevisani M, et al. Protease-activated receptor 2 sensitizes the capsaicin receptor transient receptor potential vanilloid receptor 1 to induce hyperalgesia. J Neurosci. (2004) 24:4300–12. doi:10.1523/jneurosci.0414-04.2004

355. Akiba Y, Kato S, Katsube K, Nakamura M, Takeuchi K, Ishii H, et al. Localization of TRPV1 and contractile effect of capsaicin in the vanilloid receptor (VR1) in the gastrointestinal tract. J Comp Neurol. (2005) 483:386–90. doi:10.1002/jcn.20512

356. Hofmann AF. Detoxification of lithocholic acid, a toxic bile acid: relevance to drug hepatotoxicity. Drug Metab. Rev. (2004) 36:703–22. doi:10.1081/dmr-200035475

357. Tian J, Huang S, Sun S, Ding L, Zhang E, Huang W. Bile acid signaling and bariatric surgery. Liver Res. (2017) 1:208–13. doi:10.1016/j.jliver.2017.12.007

358. Bohlen CJ, Priel A, Zhou S, King D, Siemens J, Julius D. The capsaicin receptor: a heat-activated ion channel in the pain pathway. Nature (1997) 398:816–24. doi:10.1038/39807

359. Dey T, Genoway JR, Banerjee S, Banerjee SJ. The role of transient receptor potential channels in metabolic syndrome. Hypertens Res. (2008) 31:1989–95. doi:10.1210/hyp.31.1989
mouse large intestine: high abundance and sensitivity in rectum and distal colon. Am J Physiol Gastrointest Liver Physiol. (2009) 297:C348–60. doi: 10.1152/ajpgi.90578.2008.

377. Wang P, Yan Z, Zhong J, Chen J, Ni Y, Li L, et al. Transient receptor potential vanilloid 1 activation enhances gut glucagon-like peptide-1 secretion and improves glucose homeostasis. Diabetes (2012) 61:2155–65. doi: 10.2337/db11-1503.

378. Smeets AJ, Westerterp-Plantenga MS. The acute effects of a lunch containing capsaicin on energy, plasma insulin levels and insulin binding in dog models. Phytother Res. PTR (2001) 15:391–4. doi: 10.1002/ptr.750.

379. Smeets AJ, Westerterp-Plantenga MS. The acute effects of a lunch containing capsaicin on energy and substrate utilisation, hormones, and satiety. Eur J Nutr. (2009) 48:229–34. doi: 10.1007/s00394-009-0006-1.

380. Lee E, Jung DY, Kim JH, Patel PR, Hu X, Lee Y, et al. Transient receptor potential vanilloid type-1 channel regulates diet-induced obesity, insulin resistance, and leptin resistance. FASEB J. (2015) 29:1882–92. doi: 10.1096/fj.14-268300.

381. Motter AL, Ahern GP. TRPV1-null mice are protected from diet-induced obesity. FEBS Lett. (2008) 582:2257–62. doi: 10.1016/j.febslet.2008.05.021.

382. Zhang LL, Yan Liu D, Ma LQ, Luo ZD, Cao TB, Zhong J, et al. Activation of transient receptor potential vanilloid type-1 channel prevents adipogenesis and obesity. Circ Res. (2007) 100:1063–70. doi: 10.1161/01.RES.0000266253.84850.8b.

383. Panchal SK, Bliss E, Brown L. Capsaicin in metabolic syndrome. Nutrients (2018) 10:630. doi: 10.3390/nu10050630.

384. Derbenev AV, Zsombok A. Potential therapeutic value of TRPV1 and TRPA1 in diabetes mellitus and obesity. Semin Immunopathol. (2016) 38:397–406. doi: 10.1007/s00281-015-0529-x.

385. Greiner TU, Backhed F. Microbial regulation of GLP-1 and L-cell biology. Mol Metab. (2016) 5:753–8. doi: 10.1016/j.molme.2015.05.012.

386. Sender R, Fuchs S, Milo R. Revised estimates for the number of microbes in the human microbiome. Science (2016) 354:564–9. doi: 10.1126/science.aad4786.

387. Petersen N, Reimann F, Bartfeld S, Farin HF, Ringnalds FC, Vries RG, et al. Generation of L cells in mouse and human small intestine organoids. Diabetes (2014) 63:410–20. doi: 10.23736/s0012-1878.13-10991.

388. Smeets AJ, Westerterp-Plantenga MS. The acute effects of a lunch containing capsaicin on energy and substrate utilisation, hormones, and satiety. Eur J Nutr. (2009) 48:229–34. doi: 10.1007/s00394-009-0006-1.

389. Cani PD, Hoste S, Guiot Y, Delzenne NM. Dietary nondigestible carbohydrates promote L-cell differentiation in the proximal colon of rats. Br J Nutr. (2007) 98:32–7. doi: 10.1017/S000711450791648X.

390. Petersen N, Reimann F, Bartfeld S, Farin HF, Ringnalds FC, Vries RG, et al. Generation of L cells in mouse and human small intestine organoids. Diabetes (2014) 63:410–20. doi: 10.23736/s0012-1878.13-10991.

391. Spanoagianopoulos P, Bess EN, Carmony RD, Turner BJ, The microbial pharmacists within us: a metagenomic view of xenobiotic metabolism. Nat Rev Microbiol. (2016) 14:273–87. doi: 10.1038/nrmicro.2016.17.

392. Scott TA, Quintaneiro LE, Norvaisas P, Lui PY, Wilson MP, Leung KY, et al. Host-microbe co-metabolism dictates cancer drug efficacy in C. elegans. Cell (2017) 169:442–56.e418. doi: 10.1016/j.cell.2017.03.040.

393. Knop FK, Vilsboll T, Larsen S, Holjeb P, Volund A, Madsbad S, et al. Increased postprandial responses of GLP-1 and GIP in patients with chronic pancreatitis and steatorrhea following pancreatic enzyme substitution. Am J Physiol Endocrinol Metab. (2007) 292:E324–30. doi: 10.1152/ajpendo.00598.2006.

394. Elrichmann M, Kapelle M, Ritter PR, Holst JJ, Herzeg K-H, Schmidt WE, et al. Orlistat inhibition of intestinal lipase acutely increases appetite and attenuates postprandial glucagon-like peptide-1 (7–36)–Amide 1, Cholecystokinin, and Peptide YY Concentrations. J Clin Endocrinol Metab. (2008) 93:3995–8. doi: 10.1210/jc.2008-0924.

395. Knop FK, Vilsboll T, Larsen S, Holjeb P, Volund A, Madsbad S, et al. Increased postprandial responses of GLP-1 and GIP in patients with chronic pancreatitis and steatorrhea following pancreatic enzyme substitution. Am J Physiol Endocrinol Metab. (2007) 292:E324–30. doi: 10.1152/ajpendo.00598.2006.

396. Rahat-Rozenbloom S, Fernandes J, Cheng J, Gloor GB, Wolever TM. The effect of glucagon-like peptide 1 in the treatment of STZ-induced diabetes in rats. Diabetes Obes Metab. (2008) 10:686–74. doi: 10.1111/j.1097-0285.2008.00762.x.

397. Petersen N, Reimann F, Bartfeld S, Farin HF, Ringnalds FC, Vries RG, et al. Generation of L cells in mouse and human small intestine organoids. Diabetes (2014) 63:410–20. doi: 10.23736/s0012-1878.13-10991.

398. Petersen N, Reimann F, Bartfeld S, Farin HF, Ringnalds FC, Vries RG, et al. Generation of L cells in mouse and human small intestine organoids. Diabetes (2014) 63:410–20. doi: 10.23736/s0012-1878.13-10991.

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