Review

Jia Nuo Feng and Tianru Jin*

Hepatic function of glucagon-like peptide-1 and its based diabetes drugs

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Abstract: Incretins are gut-produced peptide-hormones that potentiate insulin secretion, especially after food intake. The concept of incretin was formed more than 100 years ago, even before insulin was isolated and utilized in the treatment of subjects with type 1 diabetes. The first incretin, glucose-dependent insulinotropic polypeptide (GIP), was identified during later 1960's and early 1970's; while the second one, known as glucagon-like peptide-1 (GLP-1), was recognized during 1980's. Today, GLP-1-based therapeutic agents [also known as GLP-1 receptor (GLP-1R) agonists, GLP-1RAs] are among the first line drugs for type 2 diabetes. In addition to serving as incretin, extra-pancreatic functions of GLP-1RAs have been broadly recognized, including those in the liver, despite the absence of GLP-1R in hepatic tissue. The existence of insulin-independent or gut-pancreas-liver axis-independent hepatic function of GLP-1RAs explains why those therapeutic agents are effective in subjects with insulin resistance and their profound effect on lipid homeostasis. Following a brief review on the discovery of GLP-1, we reviewed literature on the exploration of hepatic function of GLP-1 and GLP-1RAs and discussed recent studies on the role of hepatic hormone fibroblast growth factor 21 (FGF21) in mediating function of GLP-1RAs in animal models. This was followed by presenting our perspective views.

Keywords: FGF21; GLP-1; GLP-1R; GLP-1RA

***Corresponding author: Tianru Jin**, Division of Advanced Diagnostics, Toronto General Research Institute, University Health Network, Toronto, ON M5G 1L7, Canada; Department of Physiology, Temerty Faculty of Medicine, University of Toronto, Toronto, ON M5G 1L7, Canada; and Banting and Best Diabetes Centre, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, M5G 1L7, Canada, E-mail: tianru.jin@utoronto.ca. https://orcid.org/0000-0002-0307-7391

Jia Nuo Feng, Division of Advanced Diagnostics, Toronto General Research Institute, University Health Network, Toronto, ON, Canada; Department of Physiology, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada; and Banting and Best Diabetes Centre, Temerty Faculty of Medicine, University of Toronto, Toronto, ON, Canada

The incretin hormones GIP and GLP-1

In 1902, two British scientists, Bayliss and Starling, published their milestone investigations with the utilization of experimental dogs, showing that digestive system acid infusion induced pancreatic islet secretion even after the nervous system to the intestine was blocked [1]. This observation led them to propose that the signal from the gut of the dog to its pancreas for stimulating pancreatic secretion is a chemical stimulus in nature, not necessarily involving the nervous system [1]. To further test this hypothesis, they collected extract from the intestinal wall following acid stimulation in the experimental dog and such extract was then injected into the bloodstream. Again, they detected the pancreatic "juices" following the injection. They hence concluded that such intestinal extract must contain "something" that is released from the intestine into the circulation, stimulating pancreatic islet secretion. They named such yet to be isolated biological molecules as secretin [1]. In 1905, in his Croonian Lecture, Starling also created the word "hormone", referring to factor that is released from one site but exerts its physiological or pathophysiological functions elsewhere and remotely. This marked the establishment of the new subject of physiological sciences: Endocrinology. The investigation on gut-mediated pancreatic secretion, hence, was ahead of the isolation and the utilization of insulin in the treatment of subjects with type 1 diabetes by Banting and colleagues in 1921. The nomenclature "incretin", was then coined by the Belgium scientist Jean La Barre in 1932 [2]. Originally, it refers to any gut-produced hormone or polypeptide that, under physiological conditions, can stimulate or contribute to the stimulation of the secretion of pancreatic hormones including insulin, glucagon, pancreatic polypeptide (PP), and pancreatic somatostatin. Practically, especially in the field of drug development, the focus of incretin is restricted to two such metabolic hormones known as gastric inhibitory polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) (please see below), which facilitate insulin secretion, mainly following food intake [3].

Further advancement on the concept of incretin and its bench work investigations became possible following the technique development on quantitatively measurement of

insulin in both human subjects and experimental animals with radioimmunoassay [4], followed by the development and advancement of enzyme-linked immunosorbent assays (ELISA). It is worth mentioning that unlike insulin, glucagon, and several other metabolic hormones, plasma GLP-1 level measurement is not among standard clinical biochemical tests yet. In adults, the fasting plasma GLP-1 level is usually within the range of 5–10 pmol/L [5], whereas the plasma insulin levels are almost 10-fold higher, ranging from 5–15 μU/mL, equivalent to 30–100 pmol/L [6].

The first incretin was identified by Canadian scientist Brown and colleagues during later 1960's and early 1970's [7–11]. Initially, Brown's team in University of British Columbia aimed to search for factors expressed in the duodenum that mediate the cause of increased motor activity in the stomach [10, 11]. In 1971, Brown and Dryburgh reported their isolation of a polypeptide from the intestinal mucosa with deduced amino acid sequence [8]. They named this 42-amino acid peptide as GIP as its exogenous injection reduced gastric acid secretion in experimental dogs. Two years later, Dupre, a clinical faculty member at McGill University, in collaboration with Brown and others, demonstrated that this polypeptide also possesses insulinotropic function and demonstrated the glucose-dependent nature of its insulinotropic action [12]. They renamed this hormone as glucose-dependent insulinotropic peptide, retaining the acronym GIP [12].

GIP is produced by intestinal endocrine K cells, predominantly in the duodenum. Although we haven't seen the final success on developing GIP-based therapeutic agents for diabetes or other metabolic disorders, extensive bench work studies on this hormone and its receptor (GIPR) have contributed to the development of dipeptidyl peptidase-4 (DPP-4) inhibitor sitagliptin as a diabetes drug by Merck & Co. in 2006. The knowledge advancement also contributes to the development of dual agonists that target both GIPR and GLP-1 receptor (GLP-1R). The first one, known as Tirzepatide, is now sold under the brand names Mounjaro and Zepbound, the product of Eli Lily Co. [4, 13–15]. Another such dual agonist, RG7697, developed by Novo Nordisk, has also entered the clinical trials [16], pending its final approval [14].

In 1983, a study presented by Creutzfeldt's team indicated the existence of the 2nd incretin. They assessed the action of watery rat gut extracts on glucose-induced insulin secretion before and after removal of GIP by immune-absorption [17]. The neutralization of GIP activity with the anti-GIP-specific antibody reduced the incretin effect approximately 30 %, while completely removal of GIP resulted in 50 % loss of the incretin effect [17]. During the same period, the gene that encodes the notable catabolic hormone glucagon by pancreatic α-cells, namely the proglucagon gene (*GCG*/*Gcg*), was isolated from fish, rodent species, and humans, by Habener's team in Harvard University and Bell's team in University of Chicago, respectively [18–22]. The analysis of those cDNAs suggested that human *GCG* and rodent *Gcg* encode not only glucagon but also two glucagon-like peptide hormones, namely GLP-1 and glucagon-like peptide-2 (GLP-2). The same pre-hormone, i.e. pre-proglucagon (which contains the pro-hormone proglucagon and the signal peptide consist of 20 amino acid residues), is produced in pancreatic islet α-cells, gut endocrine L cells and certain neural cells in the brainstem [23]. In contrast to GIP-producing gut endocrine K cells, gut endocrine L cells are mainly located within the ileum and colon. As shown in Figure 1, in pancreatic α-cells, the pro-hormone is processed by the pro-hormone convertase 2 (PC2) to produce the bioactive glucagon, along with glicentin-related polypeptide (GRPP), intervening peptide-1 (IP1), and major proglucagon fragment (MPGF). In the gut and brain, the same pro-hormone is processed by the pro-hormone convertase 1/3 (PC1/3) to produce active GLP-1 and GLP-2, along with glicentin, intervening peptide-2 (IP2), and oxyntomodulin. Figure 1 also shows the comparison of amino acid residues of GLP-1 (GLP-$1_{7–37}$ as an example), GLP-2, and glucagon.

In 1985, Creutzfeldt's team reported that it is GLP-1 instead of GLP-2 that can stimulate insulin secretion from isolated rat pancreatic islets [24]. A more thoroughly investigation was then presented by Mojsov, Weir, and Habener in 1987, showing that GLP-$1_{7–37}$ is a potent stimulator of insulin release in the perfused rat pancreas [25]. Nevertheless, we now generally accepted that both GLP-$1_{7–37}$ and GLP-$1_{7–36\ amide}$ are active incretin hormone in our human bodies [25–28]. Human studies on the insulinotropic action of GLP-1 were then rapidly conducted in United States of America and European countries [29–32]. The identification of GLP-1R and the generation of GLP-1R knockout mice and other related tools by Drucker's team and by others [33] have been facilitating functional studies on this gut hormone during the past decades, contributing to the development and further advancement of GLP-1-based diabetes drugs, as well as their clinical applications.

As shown in Figure 2, in pancreatic β-cells, GLP-1 or GLP-1RAs facilitates insulin secretion, involving protein kinase A (PKA) and exchange protein directly activated by cAMP (Epac) signaling. In addition, GLP-1 or GLP-1RAs can also stimulate β-cell proliferation and inhibit β-cell apoptosis. Those functions involve protein kinase B (PKB/Akt), canonical Wnt, and other signaling cascades, as well as the downstream target genes including the pro-insulin genes and the key transcription factor pancreatic duodenum homeobox-1 (PDX-1). Such classical functions of GLP-1 have already been thoroughly discussed in numerous review articles [34–37]. For current review, we will focus on hepatic function of GLP-1 and GLP-1RAs.

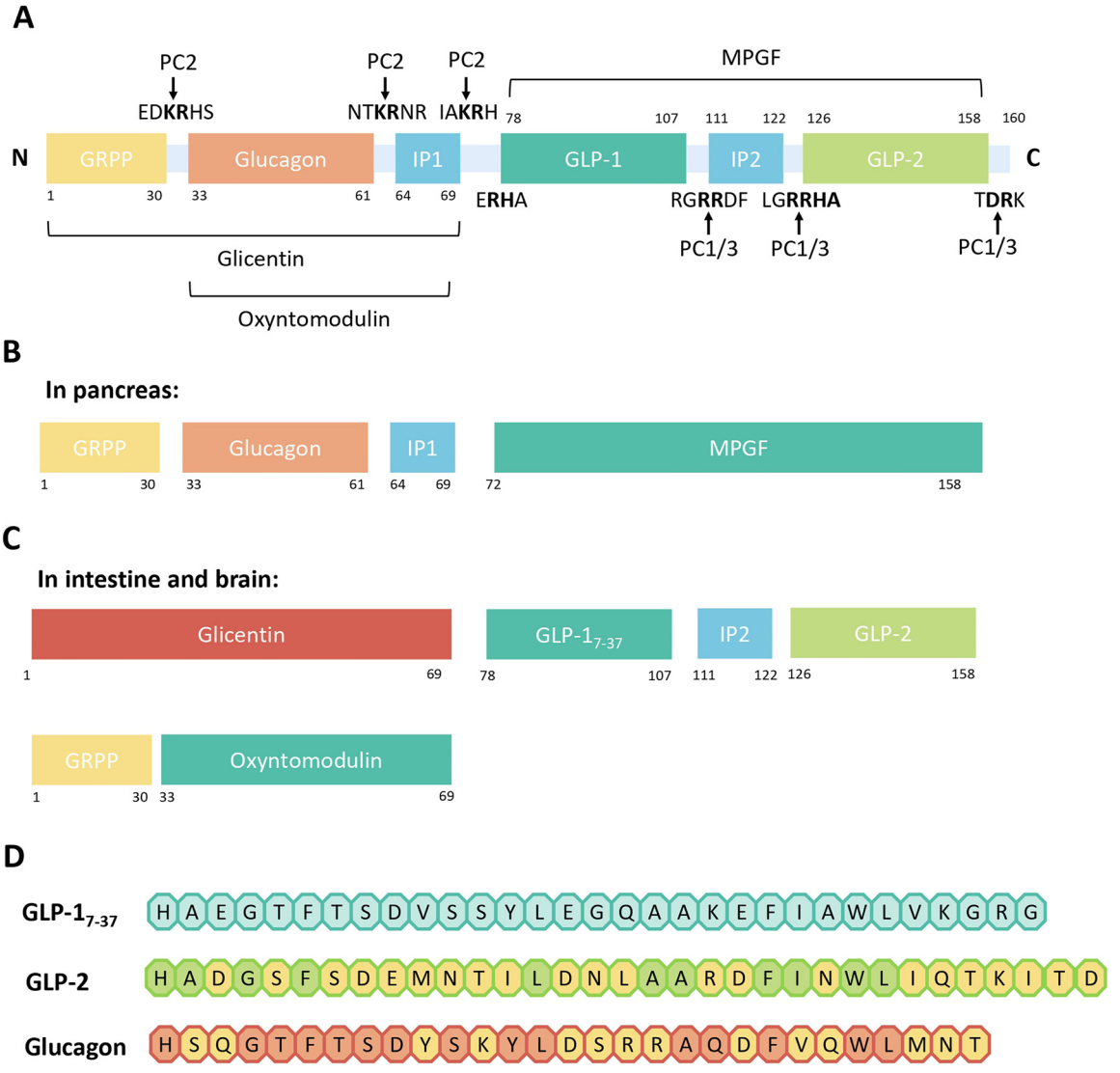

Figure 1: The structure of proglucagon (A) and its tissue-specific processing in the pancreatic islets (B) vs. that in the intestine and brain (C). (A) The structure of the prohormone proglucagon, along with the locations of three prohormone convertase 2 (PC2) and three prohormone convertase 1/3 (PC1/3) cleavage sites. (B) In pancreatic α-cells, the post translational processing, mediated by PC2, generates the products glicentin-related pancreatic polypeptide (GRPP), glucagon, intervening peptide-1 (IP1) and major proglucagon fragment (MPGF). (C) In the intestinal L cells and in the brain, the post-translational processing, mediated by PC1/PC3, generates the final products of glicentin, oxyntomodulin, glucagon-like peptide (GLP)-1, intervening peptide-2 (IP2) and GLP-2. (D) Comparison of the amino acid sequences of human GLP-1_{7-37}, GLP-2, and glucagon.

The development of GLP-1R agonists as therapeutic agents for type 2 diabetes and body weight management

Native GLP-1 molecules (both GLP-1_{7-37} and GLP-$1_{7-36\ amide}$) cannot serve as therapeutic agents *per se*, as their half-lives are very short, approximately 1.5–2.0 min, due to their rapid degradation, facilitated by ubiquitously expressed endogenous enzymes known as DPP-4 and neutral endopeptidase 24.11 (NEP 24.11). Exendin-4 (Ex-4) is a polypeptide with 39 amino acid residues isolated from the saliva of the Gila monster (*Heloderma suspectum*). Ex-4 shares 53 % of amino acid residues with mammalian GLP-1 and has been shown to mimic the function of endogenous GLP-1 on stimulating insulin secretion yet with much longer half-life (~10 min), as Ex-4 molecule is resistant to enzymatic cleavage by DPP-4. In 1992, Eng's team demonstrated the incretin function of Ex-4 in both rat and guinea pig models [38, 39]. In 1993, Goke and colleagues then demonstrated that Ex-4 is a high potency agonist while

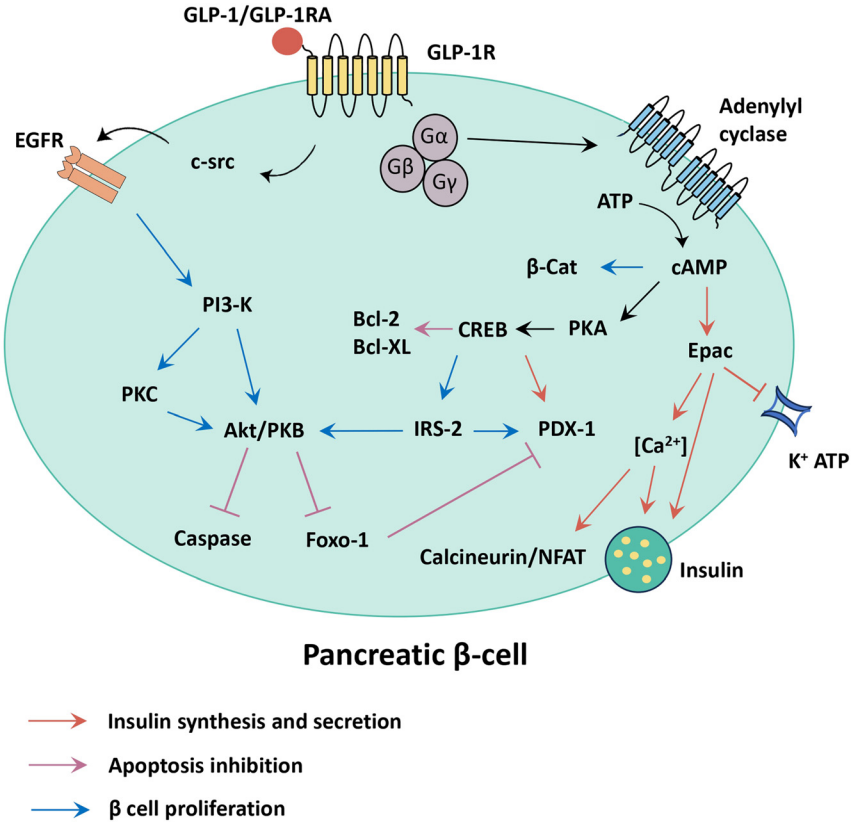

Figure 2: Function of GLP-1 and GLP-1RAs on pancreatic β-cells. In pancreatic β-cells, endogenous GLP-1 or GLP-1RA engages GLP-1R to induce downstream signaling events. This is predominantly mediated by Gα subunit that increases cAMP via the catalytic conversion of ATP by Adenylyl cyclase to activate PKA or Epac signaling. This in turn mediates the function of GLP-1/GLP-1RAs on insulin synthesis or secretion, also known as the insulinotropic effect. In addition, signaling cascades such as PKB/Akt, canonical Wnt, and others are also involved in facilitating β-cell proliferation and inhibiting apoptosis. β-cat, β-catenin; CREB, cAMP response element binding protein; Epac, exchange protein activated by 3′-5′-cyclic adenosine monophosphate [cAMP]; GLP-1, glucagon-like peptide-1; GLP-1R, GLP-1 receptor; GLP-1RA, GLP-1 receptor agonists; IRS-2, insulin receptor substrate-2; PDX-1, pancreas duodenum homeobox-1; PI3-K, phosphatidylinositol 3-kinase; PKA, protein kinase A; PKB, protein kinase B; PKC, protein kinase C.

truncated Exendin$_{9–39\ amide}$ serves as an antagonist of GLP-1-R [40]. In 1997, cDNA for the *Ex-4* gene was isolated by Chen and Drucker [41]. Chen and Drucker also analyzed the structure and expression of pancreatic and intestinal proglucagon mRNAs in the reptile *H. suspectum*. They reported the identification of two different proglucagon cDNAs in *H. suspectum*, namely lizard proglucagon I (LPI) and lizard proglucagon II (LPII), with unique 3′-untranslated regions in lizard for each of these two genes. LPI and LPII are not expressed in the lizard salivary gland while Ex-4 is not expressed in lizard pancreas or the gut [41].

A synthetic version of Ex-4, namely Exenatide, developed by Amylin Pharmaceuticals and Eli Lilly and Co., was the first GLP-1-based therapeutic agent for type 2 diabetes (T2D), approved by the Food and Drug Administration (FDA) in 2005, with the brand name Byetta®. Liraglutide, commercially known as Victoza®, is a modified version of native human GLP-1. It was approved by the European Medicines Agency (EMA) in 2009 and by the FDA in January 2010 for its clinical use. Liraglutide shares 97 % sequence identity with native human GLP-1. The non-covalent binding with the albumin molecule effectively prevents its renal elimination, making its half-life approximately 13 h [42–44]. Semaglutide, commercially known as Ozempic®, is a long-acting GLP-1RAs for T2D, approved in 2017, with a half-life of seven days [45, 46]. An equipotent once-daily oral administration form of Semaglutide (Rybelsus) was then approved in 2019 for its clinical use [47]. Table 1 presents information about brand and company names of Exenatide, Liraglutide, and Semaglutide (and its oral formulation), drug delivery methods, as well as the year they were approved by FDA for their commercial utilization.

It is worth mentioning that Liraglutide and Semaglutide are also approved for body weight management. During the past few years, the body weight lowering effect of Ozempic® has been attracting the attention world widely, making the pharmacological use of GLP-1RAs far beyond the scope of T2D. Table 1 also lists other available GLP-1RAs. Among them, Albiglutide consists of a dimer of human GLP-1 molecules fused to a recombinant albumin molecule of human origin, while Dulaglutide consists of a dimer of human GLP-1 molecules fused to a modified human immunoglobulin G4 heavy chain [48]. In addition to those listed in Table 1, Supaglutide, product of Innogen (based in Shanghai Zhang-Jiang, P.R. China), is another long-acting GLP-1RA, in which GLP-1 is fused with immunoglobulin constant region Fc fragment to prevent its enzymatic degradation [49, 50]. Additional detailed information on GLP-1 based drug development can be found in excellent review articles elsewhere [36, 51–54]. Figure 3A shows the structure of the two active native GLP-1,

Table 1: Incretin-based diabetes drugs.

Brand name	Company name	Active ingredient	FDA-approved year
GLP-1RA[a]			
Byetta	Amylin/Eli Lilly	Exenatide	2005
Bydureon	AstraZeneca	Exenatide (extended release)	2012
Victoza	Novo Nordisk	Liraglutide	2010
Saxenda	Novo Nordisk	Liraglutide	2014
Xultophy 100/3.6	Novo Nordisk	Liraglutide and insulin degludec	2016
Tanzeum	GlaxoSmithKline	Albiglutide	2014
Trulicity	Eli Lilly	Dulaglutide	2014
Adlyxin	Sanofi	Lixisenatide	2016
Soliqua 100/33	Sanofi	Lixisenatide and insulin glargine	2016
Ozempic	Novo Nordisk	Semaglutide	2017
Rybelsus	Novo Nordisk	Semaglutide (oral)	2019
Wegovy	Novo Nordisk	Semaglutide	2021
Mounjaro	Eli Lilly	Tirzepatide (GIP and GLP-1RA)	2022
DPP-4 inhibitor[b]			
Januvia	Merck	Sitagliptin	2006
Janumet	Merck	Sitagliptin and metformin	2007
Janumet XR	Merck	Sitagliptin and metformin (extended release)	2012
Steglujan	Merck	Sitagliptin and ertugliflozin	2017
Onglyza	AstraZeneca	Saxagliptin	2009
Kombiglyze XR	Bristol-Myers Squibb	Saxagliptin and metformin (extended release)	2010
Qtern	AstraZeneca	Saxagliptin and dapagliflozin	2017
Qternmet XR	AstraZeneca	Saxagliptin, dapagliflozin and metformin (extended release)	2019
Tradjenta	BOE[c]/Eli Lilly	Linagliptin	2011
Jentadueto	BOE/Eli Lilly	Linagliptin and metformin	2012
Jentadueto XR	BOE/Eli Lilly	Linagliptin and metformin (extended release)	2016
Glyxambi	BOE/Eli Lilly	Linagliptin and empagliflozin	2015
Trijardy XR	BOE/Eli Lilly	Linagliptin, empagliflozin and metformin	2020
Nesina	Takeda	Alogliptin	2013
Kazano	Takeda	Alogliptin and metformin	2013
Oseni	Takeda	Alogliptin and pioglitazone	2013

[a]Subceutaneous injection for all, except oral administration for Rybelsus, [b]oral administration for all, [c]BOE, Boehringer Ingelheim pharmaceuticals.

known as GLP-$1_{7–37}$ and GLP-$1_{7–36\ amide}$, along with the structure of two C-terminal fragments, the nonapeptide GLP-$1_{28–36\ amide}$ and the pentapeptide GLP-$1_{32–36\ amide}$. In various mouse models, these two C-terminal peptides of GLP-1 have been demonstrated to possess beneficial metabolic functions in the liver, pancreatic islets, and elsewhere. They do not require the participation of GLP-1R in exerting their functions [55–58]. Figure 3B shows the structural comparison of three major GLP-1RA therapeutic agents, i.e. Exenatide, Liraglutide, and Semaglutide, as well as that of the dual agonist Tirzepatide.

Various forms of GLP-1RAs are serving as externally provided incretin molecules with longer half-lives, administrated mainly via subcutaneous injection, except for Rybelsus® (the oral version of Semaglutide). Scientists have also developed another strategy for incretin-based drug development with success: the prevention of the degradation of endogenous GLP-1 and GIP within our body. The compound Sitagliptin, previously known as MK-0431, was demonstrated via the high throughput screening approach, to competitively inhibit the enzymatic activity of DPP-4, but not other members of the serine peptidase family. It was approved by the FDA as a T2D therapeutic agent in 2006, with the brand name Januvia®. As orally administrated T2D drug, Januvia® has also been formulated in combination with metformin, known as the drug Janumet (Table 1). Table 1 also listed other Januvia® related DPP-4 inhibitors that are approved by the FDA as T2D drugs. For additional information on DPP-4 inhibitors, please see excellent review articles elsewhere [59–62].

Controversy on GLP-1R expression in the liver

During the past two decades, substantial controversies exist in literature regarding GLP-1R expression in extra-pancreatic organs, including that in the liver, heart, adipose tissues [13, 63–65], and various immune cells [66–68]. Nevertheless, profound *in vivo* effects of native GLP-1 and GLP-1RAs in the liver as well as other extra-pancreatic organs have been reproducibly demonstrated in various experimental animal models and in clinical studies [63–65, 69–71].

Rat GLP-1R cDNA was initially isolated by Thorens in 1992 [72]. The receptor consists of 463 amino acid residues and contains seven transmembrane domains. Human GLP-1R also contains 463 amino acid residues and there is 90 % sequence homology between the two species [73]. GLP-1R is a member of the glucagon receptor family of G-protein-coupled receptors (GPCRs). The first GLP-1R knockout mouse line was created by

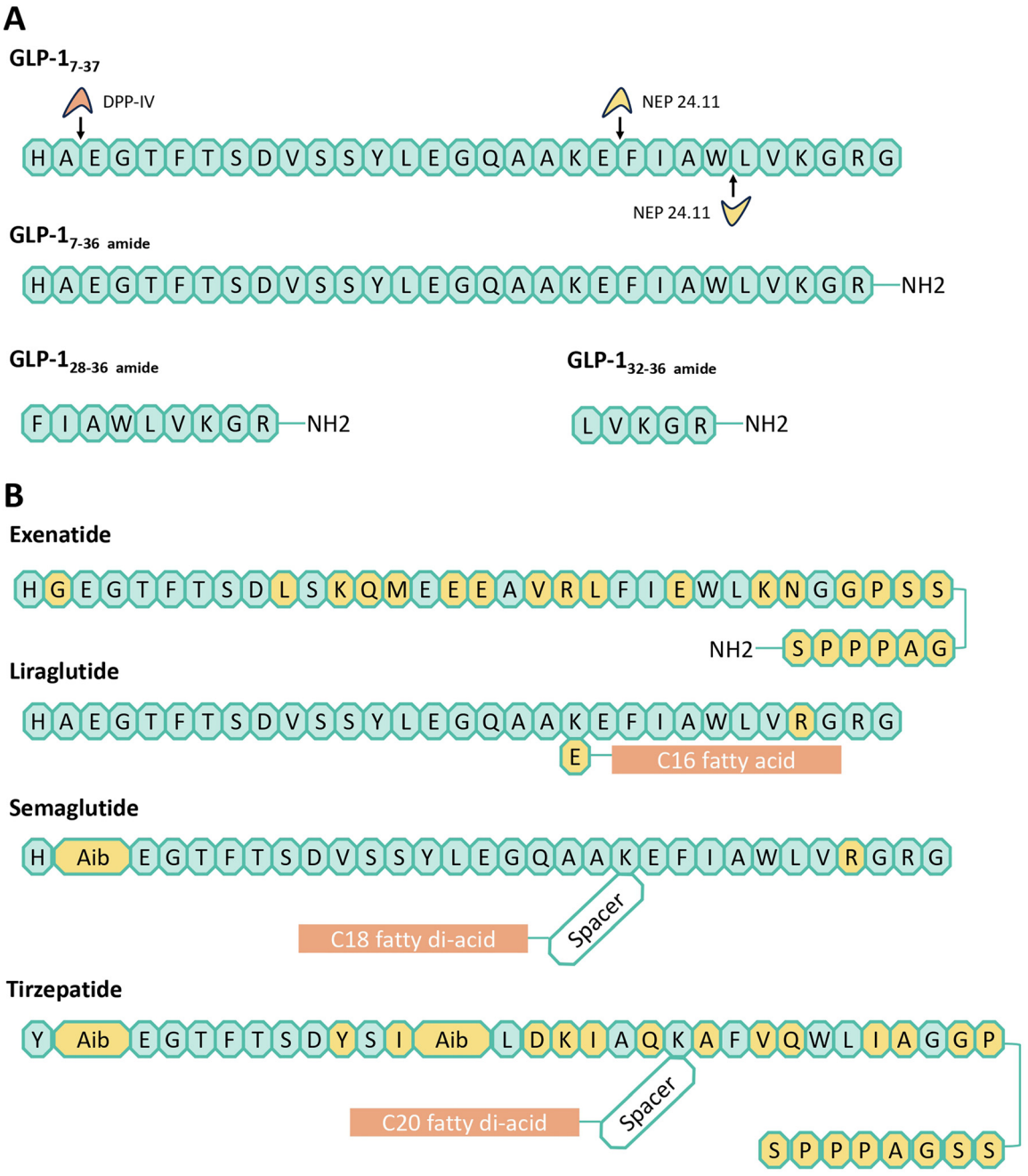

Figure 3: Structures of the active GLP-1 molecules, two C-terminal fragments, and representative incretin-based drugs. (A) The primary amino acid sequence of native human GLP-$1_{7–37}$ and GLP-$1_{7–36\ amide}$, and that of GLP-$1_{28–36\ amide}$ and GLP-$1_{32–36\ amide}$. (B) Chemical structure of Exenatide, Liraglutide, Semaglutide and Tirzepatide, with the indication of structure modifications and amino acid residue substitutions that prevent DPP-4-mediated degradation.

Scrocchi et al. in 1996 in Drucker's team [33], which has been a valuable tool for functional studies on GLP-1 and its based drugs for decades. In 1994, Campus and colleagues investigated *Glp1r* expression in mice with a combination of Northern blotting and RT-PCR. They reported the detection of *Glp1r* in small and large intestines, pancreas, and kidney [74]. Wei and Mojsov then assessed GLP-1R expression in various human tissues, claimed that mRNA isolated from the brain, heart and pancreatic islets have the same deduced amino acid sequence [75]. In 1996, with more specific approaches including *in situ* hybridization and RNase protection assay, along with regular RT-PCR, Bullock et al. in Habener's team reported the detection of rat *Glp1r* in the gastric pit of the stomach, crypts of the duodenum, pancreatic islets, and large-

nucleated cells in the lung. They, however, cannot detect *Glp1r* signal in the liver, skeletal muscle, kidney, heart, or adipocytes [76]. They suggested the GLP-1R expressed in the kidney and heart might be structural variants of the known receptor [76]. Twenty-eight years passed, such second GLP-1 receptor postulation, however, has not been proved or disproved yet. It is worth mentioning that expression of GLP-1R in the lung is very abundant, triggered extensive investigations on repurposing GLP-1-based drugs in the treatment of acute as well as chronic lung injury, as we and others have studied and reviewed recently [77–82].

Due to the profound hepatic function of GLP-1 and its based diabetes drugs, great efforts have been made in the determination that whether GLP-1R is expressed in hepatocytes. A few laboratories reported the detection of *Glp1r* mRNA and GLP-1R protein in human as well as mouse hepatic cell lines and in the mouse liver [69, 83, 84], in contrast with the early report by Bullock and colleagues [76]. Investigations by Panjwani et al. and by Baggio et al. in Drucker's team suggested that the controversy is at least partially due to the lack of reliable anti-GLP-1R antibodies [63, 64]. With the nonbias RNA-seq approaches, we and others have shown that mouse or human liver do not express mRNA that encodes mouse or human GLP-1R [63, 70, 71].

Certain relatively reliable GLP-1R antibodies (3F52 for humans and 7F38 for mice) have been generated by Knudsen's team, which can be utilized in the detection of GLP-1R expression by immunohistochemistry (IHC) [85, 86]. These new antibodies cannot detect GLP-1R signaling in either human or mouse liver. Taken together, with the effort by several research teams as well as via technical advancement on bulk and single-cell RNA-seq approaches [63, 87], we now commonly accept that human and mouse hepatocytes do not express GLP-1R [63–65, 77]. In adipose tissues, only a very small portion of cells that express detectable *Glp1r*, which are endothelial or hematopoietic origin. GLP-1R is also expressed in intestinal αβ and γδ T lymphocytes [88].

Hepatic function of GLP-1R agonists could be independent of the gut-pancreas-liver axis

Physiologically, the incretin hormone GLP-1 is released from the gut endocrine L cells postprandially, capable of augmenting pancreatic insulin secretion. Liver is among important metabolic organs which mediate function of insulin on both glucose and lipid homeostasis. Hence, the three organs form the important gut-pancreas-liver axis, orchestrating energy homeostasis at daily bases. During the past three decades, however, extensive investigations have revealed various extra-pancreatic functions of GLP-1 and the therapeutic agents GLP-1RAs [36, 89]. Indeed, function of GLP-1 and GLP-1RAs on the inhibition of food intake and gastric empty, on cardiac protection, anti-inflammation, renal protection, immune regulation, as well as their insulin mimetic effect, are far beyond the scope of serving solely as an incretin hormone. For decades, scientists have paid close attention in the determination of hepatic function of GLP-1 and GLP-1RAs, exploring the existence of insulin-independent or gut-pancreas-liver axis-independent, direct hepatic function of them. A fundamental scientific question arises: if metabolic functions of GLP-1RAs entirely rely on the gut-pancreas-liver axis or insulin, how can we explain the profound therapeutic effects of GLP-1RAs in human subjects and animal models with severe insulin resistance? Furthermore, the pure incretin effect on improving glucose disposal cannot effectively explain the profound effect of GLP-1RAs on lipid homeostasis, especially in insulin resistance state, as demonstrated by Adeli and his colleagues during the past two decades [90–92]. Although we can attribute the insulin signaling sensitization effect of GLP-1RAs to their anti-inflammation features, it is still possible that GLP-1RAs can exert gut-pancreas-liver axis-independent or insulin-independent hepatic function, as we and others have discussed [13, 93, 94].

During early days, a few investigations have suggested that GLP-1 can improve glucose tolerance by stimulating insulin release, facilitating insulin sensitivity, and by increasing insulin-independent glucose disposal [28, 30, 31]. Mechanistically, how these multiple functions are achieved remains largely unexplored. In 2013, a further clinical investigation with healthy human subjects was conducted by Ferrannini's team in Pisa, Italy, with the utilization of pancreatic clamp approach to maintain plasma insulin and glucagon at consistent levels. In such experimental settings, they showed that infusion of physiological levels of native GLP-1 led to 27 % reduction in endogenous-hepatic glucose production (EGP). As GLP-1 inhibited endogenous glucose production under conditions where plasma insulin and glucagon levels were stable, such inhibition can only be attributed to either a direct inhibitory effect of GLP-1 on hepatic glucose production (gluconeogenesis); or an event that is mediated by the central nervous system, via organ-organ communications [95].

Since GLP-1R is not expressed in the liver, how can native GLP-1 as well as GLP-1RAs exert their insulin-independent hepatic function on reducing EGP as well as on improving lipid

homeostasis? In addition to the suggestion on the involvement of central nervous system or organ-organ communications, several investigations have shown that the C-terminal fragments of GLP-1, known as GLP-$1_{28–36\ amide}$ and GLP-$1_{32–36\ amide}$, may exert direct function on the liver, pancreatic islets, and elsewhere, without the participation of GLP-1R [55–58]. Such theory, supported by observations made by Habener's team and our team, and by others [55–58], however, cannot explain pharmacological hepatic function of GLP-1RAs as those drugs are resistant to DPP-4-mediated cleavage, and may not be able to produce such C-terminal short peptides. Thus, we need to further broaden our view on organ-organ communications in understanding hepatic functions of GLP-1RAs. The communications may involve other metabolic hormones.

As presented in Figure 4, hepatic function of GLP-1 can be executed by the well-defined gut-pancreas-liver axis, which is dependent on the feature of GLP-1 as an incretin, mediated by insulin. The existence of gut-pancreas-liver axis independent hepatic function of GLP-1 has attracted our attention for decades. This could be mediated by a direct effect of C-terminal fragments of GLP-1. As GLP-1R is not expressed in the liver, hepatic function of GLP-1 and GLP-1Rs likely involves GLP-1R expressed in an extra-hepatic organ, via more complicated organ-organ communications.

The involvement of the hepatic hormone FGF21

Among more than two dozen of peptide hormones recognized during the past half-century [96], fibroblast growth factor 21 (FGF21) is an important one and it is mainly produced by hepatocytes [96, 97]. Plasma FGF21 level rises during fasting [98–102], making it a "starvation" hormone [103–105]. Extensive studies have revealed that FGF21 induction after fasting occurs via the activation of the nuclear receptor peroxisome proliferator-activated receptor α (PPARα) [98, 99, 106–108]. During the starvation response period, the PPARα/FGF21/PGC-1α axis facilitates fatty acid oxidation, tri-carboxylic acid cycle flux and gluconeogenesis [109]. A battery of clinical trials has demonstrated that the two most promising beneficial metabolic effects of FGF21 analogs are the improvement on insulin signaling and plasma lipid profiles [97, 110]. More integrated studies have also defined FGF21 as a "stress" hormone. Its plasma level and hepatic production can be up regulated by high fat diet (HFD) challenge; low carbohydrate ketogenic diet consumption; protein, or amino acid restriction; fasting; as well as fructose or alcohol intake [97, 111–114]. Importantly, function of FGF21 on glucose and lipid homeostasis, such as

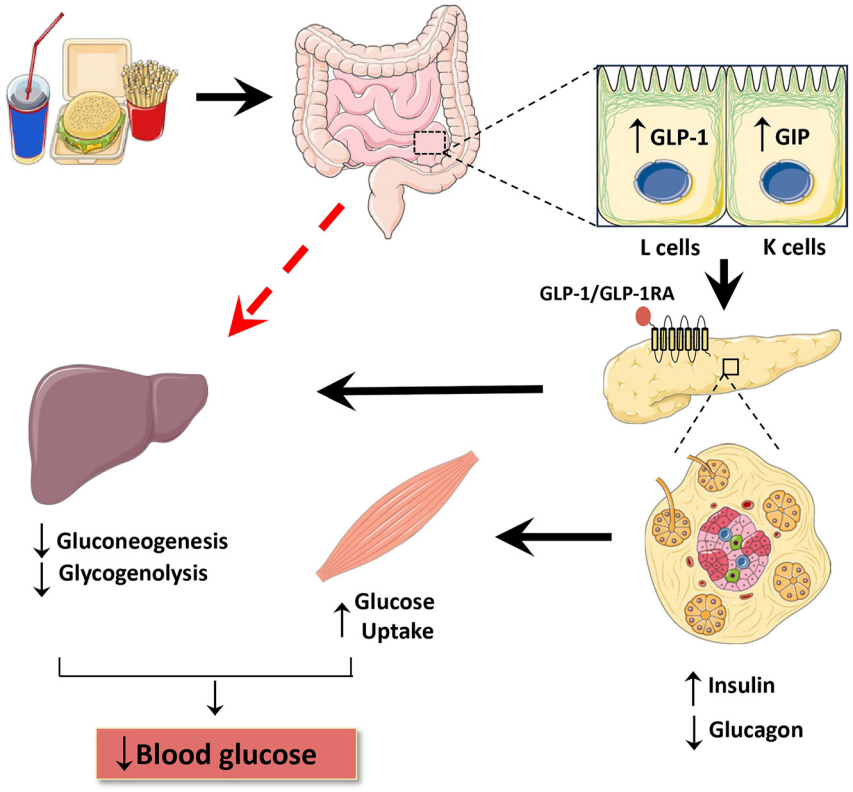

Figure 4: Illustration of gut-pancreas-liver axis dependent and independent hepatic function of GLP-1 and GLP-1RAs. Postprandially released glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP) (from the gut) facilitate insulin secretion from pancreatic islets. Insulin targets the liver (and other organs, with muscle as an example only), resulting in reduced gluconeogenesis and glycogenolysis. These, along with reduced glucagon release by pancreatic α-cells, collectively contribute to improved glucose disposal. Such gut-pancreas-liver axis dependent function of GLP-1 and GLP-1 receptor agonists (GLP-1RAs) attributes to their feature as the incretin, indicated with the heavy black arrows. The existence of the incretin feature-independent function of GLP-1 and GLP-1RAs (indicated with the dotted heavy red arrow) explains the therapeutic capacity of GLP-1RAs in type 2 diabetes (T2D) patients with insulin resistance, and their profound effect on lipid homeostasis.

the facilitation of fatty acid oxidation, is independent of insulin function. Notably, FGF21 has been shown to mediate functions of hyperlipidemia drugs such as fibrates.

In 2012, Yang and colleagues reported that in HFD-challenged *ApoE*$^{-/-}$ mice, along with adenovirus-mediated adiponectin knockdown, Liraglutide treatment stimulated hepatic *Fgf21* expression. They revealed that in mice, hypoadiponectinemia results in increased insulin resistance, associated with increased hepatic *Fgf21* expression, and reduced hepatic *Fgfr1* and *Klb* levels [115]. *Fgfr1* and *Klb* encode the FGF21 receptor (fibroblast growth factor receptor 1, FGFR1) and the obligatory co-receptor β-klotho (KLB) [115]. We now generally accept the view that reduced expression of *Fgfr1* and *Klb* represents the state of FGF21 resistance [114]. Liraglutide treatment improved insulin sensitivity and further increased hepatic *Fgf21* expression [115]. The indirect effect of GLP-1RA activation on attenuating hepatic triglyceride (TG) level in HFD-fed *ApoE*$^{-/-}$ mice, however, was reported by Panjwani and colleagues in Drucker's team in 2013, utilizing the GLP-1RA Taspoglutide [64]. Lee and colleagues reported that 10-week Ex-4 treatment in HFD-fed mice attenuated the development of hepatic steatosis, associated with increased hepatic FGF21 expression [116]. Nonogaki et al. observed that in individually housed KKAy mice, Liraglutide treatment reduced food intake, body weight, as well as blood glucose level, in the absence of appreciable changes on insulin or glucagon levels. Notably, the treatment increased both plasma FGF21 hormone level and hepatic *Fgf21* mRNA expression [117]. In the assessment of mouse liver cytokine profiles, Liu and colleagues demonstrated the stimulatory effect of Ex-4 treatment on FGF21 in the diabetic *db/db* mouse model. In both *db/db* mice and *Pax6*$^{m/-}$ mice, Liraglutide treatment showed the stimulatory effect on hepatic FGF21 production. Their further investigations allowed them to attribute function of hepatic FGF21 in mediating the regulatory effect of GLP-1RAs on attenuating hepatic gluconeogenesis [93].

Liu and colleagues conducted further mechanistic investigation on FGF21-mediated hepatic function of the GLP-1RA Liraglutide recently, expanding the scope to both glucose and lipid homeostasis [63]. First, they reproduced the observation, showing the stimulatory effect of Liraglutide treatment on hepatic FGF21 expression in wild type C57BL/6J mice on regular chow diet feeding. They then clarified that such stimulation does not occur in mouse primary hepatocytes *in vitro*, consistent with the lack of detection of *Glp1r* in mouse liver by RNAseq and other means. They then showed that in wild type C57BL/6J mice on HFD challenge, Liraglutide treatment but not sitagliptin treatment increased hepatic *Fgf21* level and plasma FGF21 hormone level, although the two incretin-based drugs showed comparable metabolic beneficial effect on glucose disposal, insulin sensitivity, plasma TG level and lipid homeostasis. As anticipated, Liraglutide but not sitagliptin treatment showed appreciable effect on reducing body weight gain and fat mass. It is worth mentioning that HFD challenge induced repression on *Fgfr1* and *Klb* expression can be effectively reversed by either Liraglutide or Sitagliptin treatment. Thus, although the DPP-4 inhibitor Sitagliptin cannot upregulate hepatic FGF21 production, it may improve FGF21 sensitivity in subjects with obesogenic diet challenge via a yet to be explored mechanism.

To determine whether GLP-1R expressed in an extra-hepatic organ mediates the function of Liraglutide on stimulating hepatic FGF21 production via organ-organ communications, Liu and colleagues then tested the effect of Liraglutide treatment in HFD-challenged *GLP-1R*$^{-/-}$ mice [63]. Following 12 weeks of HFD consumption, *GLP-1R*$^{-/-}$ mice showed increased body weight, moderately impaired glucose disposal but comparable plasma and hepatic TG levels, when compared with the same knockout mice on chow diet feeding. Those observations aligned with the previous report that *GLP-1R*$^{-/-}$ mice are moderately resistance to HFD challenge [118]. Nevertheless, the body weight lowering effect of Liraglutide treatment was completely lost in HFD challenged *GLP-1R*$^{-/-}$ mice. More importantly, Liraglutide treatment did not stimulate plasma FGF21 elevation or hepatic FGF21 production, regardless of LFD or HFD feeding. Thus, GLP-1R expressed in an extra-hepatic organ is required for Liraglutide to lower body weight and to stimulate hepatic FGF21 production, via yet to be revealed mechanism, involving organ-organ communications.

To further determine the effect of hepatic FGF21 in mediating the "therapeutic" effect of GLP-1RAs, Liu and colleagues tested the effect of Liraglutide treatment in liver-specific FGF21 knockout mice with obesogenic dietary challenge [63]. The body weight, liver weight and fat mass lowering effects of Liraglutide treatment, observed in the control mice, were partially lost in liver-specific FGF21 knockout mice, while Liraglutide treatment was still able to improve insulin tolerance in the knockout mice, assessed by intraperitoneal insulin tolerance test. Importantly, Liraglutide treatment failed to reduce serum and hepatic TG levels in the knockout mice. Hepatic gene assessment suggested that this is likely due to the loss of upregulation of genes that are involved in fatty acid oxidation in the liver specific FGF21 knockout mice, which are among the known downstream targets and effectors of FGF21.

More recently, Le et al. reproduced the observation in mice that periphery Liraglutide treatment increases plasma FGF21 levels. Liraglutide-induced weight loss was also impaired in liver-specific FGF21 knockout mice with high carbohydrate diet or high fat high sugar diet challenge.

Importantly, they found that loss of neuronal *Klb*, which encodes the obligatory co-receptor for FGF21, attenuated Liraglutide treatment induced weight loss in mice on obesogenic dietary challenges [94].

Summary and perspectives

As presented above, extra-pancreatic functions of GLP-1 and GLP-1RAs, including those in the liver, has been recognized for about three decades. Investigations on extra-pancreatic functions of GLP-1 and GLP-1RAs have gradually broadened our view on GLP-1 as a multiple functional hormone and deepened our mechanistic understanding on how this hormone works on improving energy homeostasis via targeting multiple organs. The investigations have also further broadened the application of GLP-1RAs in disease treatment, including body weight management and metabolic dysfunction-associated fatty liver disease (MAFLD) (previously known as non-alcoholic fatty liver disease, NAFLD) [63, 119].

Regarding hepatic function of GLP-1 and GLP-1RAs, in addition to the well-defined gut-pancreas-liver axis, the central dogma on function of incretin, the insulin-independent hepatic function of GLP-1 and GLP-1RAs exist, and such function involves the hepatic hormone FGF21. As presented in Figure 5,

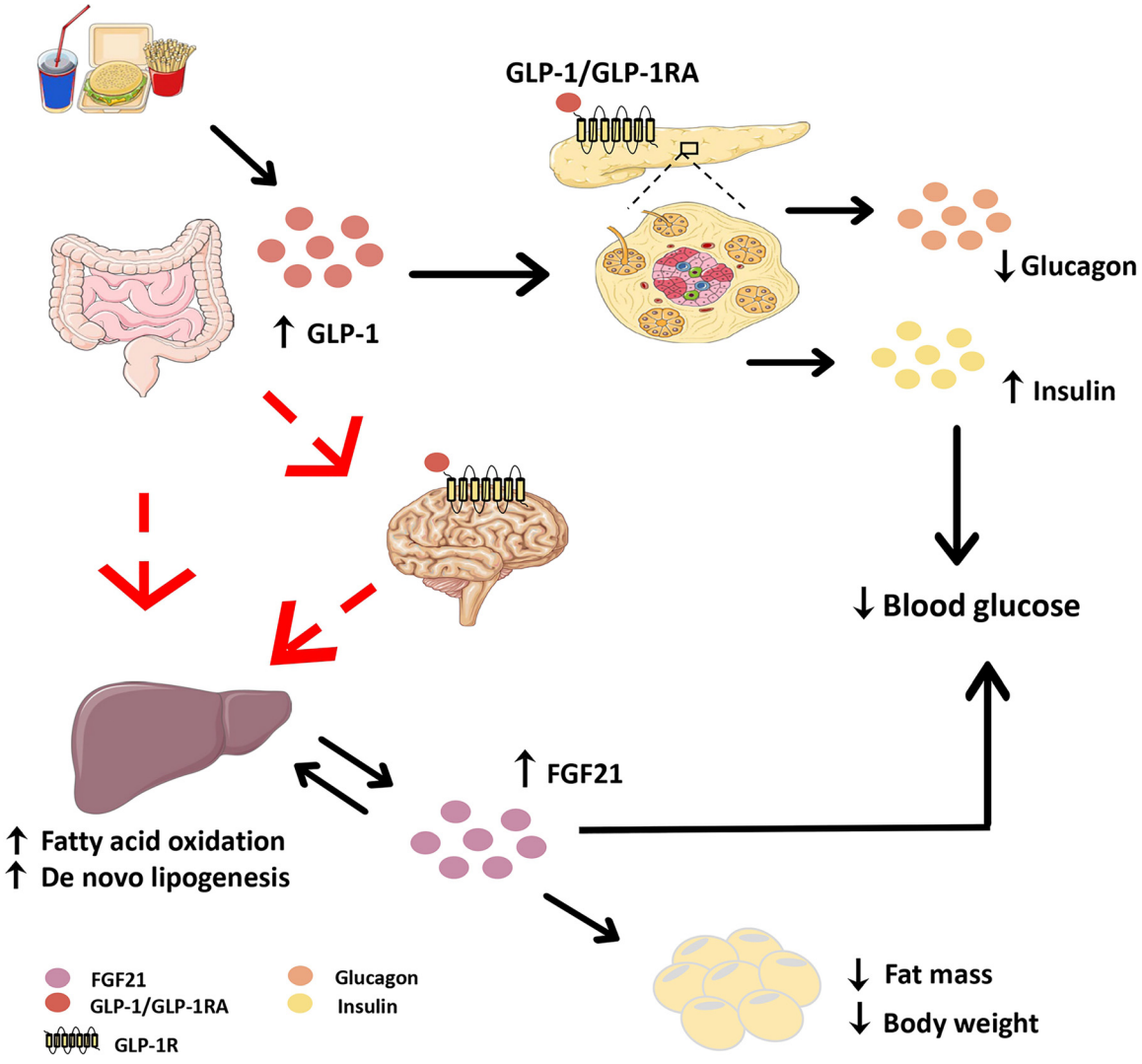

Figure 5: Hepatic FGF21 as a link for GLP-1RAs in exerting their incretin feature-independent hepatic function. Postprandially released glucagon-like peptide-1 (GLP-1) or GLP-1 receptor agonists (GLP-1RAa) administration targets pancreatic islets for facilitating glucose disposal (indicated with black arrows). GLP-1 and GLP-1RAs may target the liver directly, or via a gut-brain-liver axis (indicated with dotted red heavy arrows), leading to the regulation of hepatic fibroblast growth factor 21 (FGF21) production and the improvement of FGF21 sensitivity. Such hormonal factors and neuronal system mediated organ-organ communications explain the capability of GLP-1 and GLP-1RAs in targeting multiple organs (with liver and adipose tissues as examples), which may or may not express the GLP-1R molecules.

postprandial GLP-1 release or GLP-1RA administration leads to increased insulin secretion and reduced glucagon secretion by pancreatic islets, which contribute to improved glucose disposal and reduced hepatic gluconeogenesis. Gut GLP-1 release or GLP-1RA administration can also target the liver via yet to be further explored mechanisms, either via directly targeting the liver, without involving the current recognized eponymous receptor; or via GLP-1R expressed in an extra-hepatic organ. Considering that profound body weight lowering effect of GLP-1RAs are mediated by targeting brain GLP-1R signaling, it is reasonable to propose the existence of gut-brain-liver axis that mediating function of GLP-1RA on the liver, involving the regulation of FGF21 production and its sensitivity [63, 94].

In the near future, we anticipate that great effort will be made to determine mechanistically how brain GLP-1R signaling mediates the insulin-independent hepatic function of GLP-1RAs, including the upregulation of hepatic FGF21 production and sensitivity. The relationship between FGF21 sensitivity and insulin sensitivity is another fundamentally question for physiological scientists to address. Furthermore, adipose tissues are known to produce FGF21, exerting its function in paracrine and autocrine manners, while GLP-1R expression can only be detected in a small portion of cells in the adipose tissue that are hematopoietic and endothelial origins. Whether brain GLP-1R also mediates function of GLP-1 and GLP-1RAs via a gut-brain-adipose tissue axis, involving adipose tissue FGF21 production and function, is worth to be explored. Indeed, FGF21 has been shown to regulate white adipose tissue browning, associated with increased thermogenesis [120], while in the mouse model, such events were shown to be required for maximal weight loss in GLP-1 therapy [121]. It is also worth mentioning that efforts have been made to develop GLP-1 and FGF21 dual agonists with the success in preclinical investigations [122–125]. Whether this line of research will lead to new therapeutic agents for diabetes and other metabolic disorders is worth watching.

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References

1. Bayliss WM, Starling EH. The mechanism of pancreatic secretion. J Physiol 1902;28:325–53.
2. Barre L. Sur les possibilities d’un traitement du diabete par l’incretine. Bull Acad R Med Belg 1932;12:620–34.
3. Rehfeld JF. The origin and understanding of the incretin concept. Front Endocrinol 2018;9:387.
4. Tian L, Jin T. The incretin hormone GLP-1 and mechanisms underlying its secretion. J Diabetes 2016;8:753–65.
5. Padidela R, Patterson M, Sharief N, Ghatei M, Hussain K. Elevated basal and post-feed glucagon-like peptide 1 (GLP-1) concentrations in the neonatal period. Eur J Endocrinol 2009;160:53–8.
6. Carmina E, Stanczyk FZ, Lobo RA. Chapter 34 – laboratory assessment. In: Strauss JF, Barbieri RL, editors. Yen & Jaffe’s reproductive endocrinology, 7th ed. Philadelphia: W.B. Saunders; 2014:822–50.e3 pp.
7. Brown JC. A gastric inhibitory polypeptide. I. The amino acid composition and the tryptic peptides. Can J Biochem 1971;49:255–61.
8. Brown JC, Dryburgh JR. A gastric inhibitory polypeptide. II. The complete amino acid sequence. Can J Biochem 1971;49:867–72.
9. Kieffer TJ. In memoriam-John C. Brown, PhD, DSc, FRSC, 1938–2016: discoverer of GIP and motilin. Gastroenterology 2017;153:1169–71.
10. Brown JC, Pederson RA, Jorpes E, Mutt V. Preparation of highly active enterogastrone. Can J Physiol Pharmacol 1969;47:113–4.
11. Brown JC. Presence of a gastric motor-stimulating property in duodenal extracts. Gastroenterology 1967;52:225–9.
12. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metabol 1973;37:826–8.
13. Jin T, Weng J. Hepatic functions of GLP-1 and its based drugs: current disputes and perspectives. Am J Physiol Endocrinol Metab 2016;311: E620–7.
14. Jin T. Tirzepatide, a new class of incretin-based drug for diabetes. Obes Med 2023;39:100483.
15. El K, Douros JD, Willard FS, Novikoff A, Sargsyan A, Perez-Tilve D, et al. The incretin co-agonist tirzepatide requires GIPR for hormone secretion from human islets. Nat Metab 2023;5:945–54.
16. Frias JP, Bastyr EJ 3rd, Vignati L, Tschop MH, Schmitt C, Owen K, et al. The sustained effects of a dual GIP/GLP-1 receptor agonist, NNC0090-2746, in patients with type 2 diabetes. Cell Metabol 2017;26:343–52.e2.
17. Ebert R, Unger H, Creutzfeldt W. Preservation of incretin activity after removal of gastric inhibitory polypeptide (GIP) from rat gut extracts by immunoadsorption. Diabetologia 1983;24:449–54.
18. Bell GI, Sanchez-Pescador R, Laybourn PJ, Najarian RC. Exon duplication and divergence in the human preproglucagon gene. Nature 1983;304:368–71.
19. Bell GI, Santerre RF, Mullenbach GT. Hamster preproglucagon contains the sequence of glucagon and two related peptides. Nature 1983;302:716–8.

20. Lund PK, Goodman RH, Dee PC, Habener JF. Pancreatic preproglucagon cDNA contains two glucagon-related coding sequences arranged in tandem. Proc Natl Acad Sci U S A 1982;79: 345–9.
21. Lopez LC, Frazier ML, Su CJ, Kumar A, Saunders GF. Mammalian pancreatic preproglucagon contains three glucagon-related peptides. Proc Natl Acad Sci U S A 1983;80:5485–9.
22. Heinrich G, Gros P, Lund PK, Bentley RC, Habener JF. Pre-proglucagon messenger ribonucleic acid: nucleotide and encoded amino acid sequences of the rat pancreatic complementary deoxyribonucleic acid. Endocrinology 1984;115:2176–81.
23. Jin T. Mechanisms underlying proglucagon gene expression. J Endocrinol 2008;198:17–28.
24. Schmidt WE, Siegel EG, Creutzfeldt W. Glucagon-like peptide-1 but not glucagon-like peptide-2 stimulates insulin release from isolated rat pancreatic islets. Diabetologia 1985;28:704–7.
25. Mojsov S, Weir GC, Habener JF. Insulinotropin: glucagon-like peptide I (7–37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest 1987;79:616–9.
26. Holst J, Ørskov C, Vagn Nielsen O, Schwartz T. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett 1987;211:169–74.
27. Hansen L, Deacon CF, Orskov C, Holst JJ. Glucagon-like peptide-1-(7–36)amide is transformed to glucagon-like peptide-1-(9–36)amide by dipeptidyl peptidase IV in the capillaries supplying the L cells of the porcine intestine. Endocrinology 1999;140:5356–63.
28. Orskov C, Wettergren A, Holst JJ. Biological effects and metabolic rates of glucagonlike peptide-1 7–36 amide and glucagonlike peptide-1 7–37 in healthy subjects are indistinguishable. Diabetes 1993;42:658–61.
29. Nathan DM, Schreiber E, Fogel H, Mojsov S, Habener JF. Insulinotropic action of glucagonlike peptide-I-(7–37) in diabetic and nondiabetic subjects. Diabetes Care 1992;15:270–6.
30. Gutniak M, Orskov C, Holst JJ, Ahren B, Efendic S. Antidiabetogenic effect of glucagon-like peptide-1 (7–36)amide in normal subjects and patients with diabetes mellitus. N Engl J Med 1992;326:1316–22.
31. D'Alessio DA, Kahn SE, Leusner CR, Ensinck JW. Glucagon-like peptide 1 enhances glucose tolerance both by stimulation of insulin release and by increasing insulin-independent glucose disposal. J Clin Invest 1994; 93:2263–6.
32. Deacon CF, Johnsen AH, Holst JJ. Degradation of glucagon-like peptide-1 by human plasma in vitro yields an N-terminally truncated peptide that is a major endogenous metabolite in vivo. J Clin Endocrinol Metab 1995;80:952–7.
33. Scrocchi LA, Brown TJ, MaClusky N, Brubaker PL, Auerbach AB, Joyner AL, et al. Glucose intolerance but normal satiety in mice with a null mutation in the glucagon-like peptide 1 receptor gene. Nat Med 1996;2:1254–8.
34. Brubaker PL, Drucker DJ. Minireview: glucagon-like peptides regulate cell proliferation and apoptosis in the pancreas, gut, and central nervous system. Endocrinology 2004;145:2653–9.
35. Jin T. Current understanding on role of the Wnt signaling pathway effector TCF7L2 in glucose homeostasis. Endocr Rev 2016;37:254–77.
36. Muller TD, Finan B, Bloom SR, D'Alessio D, Drucker DJ, Flatt PR, et al. Glucagon-like peptide 1 (GLP-1). Mol Metabol 2019;30:72–130.
37. Xiong X, Shao W, Jin T. New insight into the mechanisms underlying the function of the incretin hormone glucagon-like peptide-1 in pancreatic β-cells: the involvement of the Wnt signaling pathway effector β-catenin. Islets 2012;4:359–65.
38. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from Guinea pig pancreas. J Biol Chem 1992;267:7402–5.
39. Malhotra R, Singh L, Eng J, Raufman JP. Exendin-4, a new peptide from *Heloderma suspectum* venom, potentiates cholecystokinin-induced amylase release from rat pancreatic acini. Regul Pept 1992;41:149–56.
40. Goke R, Fehmann HC, Linn T, Schmidt H, Krause M, Eng J, et al. Exendin-4 is a high potency agonist and truncated exendin-(9–39)-amide an antagonist at the glucagon-like peptide 1-(7–36)-amide receptor of insulin-secreting beta-cells. J Biol Chem 1993;268:19650–5.
41. Chen YE, Drucker DJ. Tissue-specific expression of unique mRNAs that encode proglucagon-derived peptides or exendin 4 in the lizard. J Biol Chem 1997;272:4108–15.
42. Juhl CB, Hollingdal M, Sturis J, Jakobsen G, Agersø H, Veldhuis J, et al. Bedtime administration of NN2211, a long-acting GLP-1 derivative, substantially reduces fasting and postprandial glycemia in type 2 diabetes. Diabetes 2002;51:424–9.
43. Vilsbøll T, Zdravkovic M, Le-Thi T, Krarup T, Schmitz O, Courrèges JP, et al. Liraglutide, a long-acting human glucagon-like peptide-1 analog, given as monotherapy significantly improves glycemic control and lowers body weight without risk of hypoglycemia in patients with type 2 diabetes. Diabetes Care 2007;30:1608–10.
44. Drucker DJ, Dritselis A, Kirkpatrick P. Liraglutide. Nat Rev Drug Discov 2010;9:267–8.
45. Lau J, Bloch P, Schäffer L, Pettersson I, Spetzler J, Kofoed J, et al. Discovery of the once-weekly glucagon-like peptide-1 (GLP-1) analogue semaglutide. J Med Chem 2015;58:7370–80.
46. Scheen AJ. Semaglutide: a promising new glucagon-like peptide-1 receptor agonist. Lancet Diabetes Endocrinol 2017;5:236–8.
47. Lipscombe LL. In poorly controlled type 2 diabetes, oral semaglutide was noninferior to liraglutide for reducing HbA1c. Ann Intern Med 2019;171:Jc29.
48. Nauck M. Incretin therapies: highlighting common features and differences in the modes of action of glucagon-like peptide-1 receptor agonists and dipeptidyl peptidase-4 inhibitors. Diabetes Obes Metabol 2016;18:203–16.
49. Liao Y, Ma A, Wang Z, Zhou Y, Liu L, Zhang N, et al. Pharmacokinetic and pharmacodynamic studies of supaglutide in rats and monkeys. Eur J Pharmaceut Sci 2022;175:106218.
50. Cui Q, Liao Y, Jiang Y, Huang X, Tao W, Zhou Q, et al. Novel GLP-1 analog supaglutide improves glucose homeostasis in diabetic monkeys. J Endocrinol 2021;248:145–54.
51. Drucker DJ. The GLP-1 journey: from discovery science to therapeutic impact. J Clin Invest 2024;134:e175634.
52. Drucker DJ, Habener JF, Holst JJ. Discovery, characterization, and clinical development of the glucagon-like peptides. J Clin Invest 2017; 127:4217–27.
53. Hinnen D. Glucagon-like peptide 1 receptor agonists for type 2 diabetes. Diabetes Spectr 2017;30:202–10.
54. Holst JJ. The physiology of glucagon-like peptide 1. Physiol Rev 2007; 87:1409–39.
55. Ip W, Shao W, Chiang YT, Jin T. GLP-1-derived nonapeptide GLP-1(28–36)amide represses hepatic gluconeogenic gene expression and improves pyruvate tolerance in high-fat diet-fed mice. Am J Physiol Endocrinol Metab 2013;305:E1348–58.
56. Shao W, Wang Z, Ip W, Chiang YT, Xiong X, Chai T, et al. GLP-1(28-36) improves beta-cell mass and glucose disposal in streptozotocin-induced diabetic mice and activates cAMP/PKA/beta-catenin signaling in beta-cells in vitro. Am J Physiol Endocrinol Metab 2013;304: E1263–72.

57. Liu Z, Stanojevic V, Brindamour LJ, Habener JF. GLP1-derived nonapeptide GLP1(28–36)amide protects pancreatic beta-cells from glucolipotoxicity. J Endocrinol 2012;213:143–54.
58. Liu Y, Kubota S, Iizuka K, Yabe D. Cardioprotective effects of GLP-1(28–36a): a degraded metabolite or GLP-1's better half? J Diabetes Invest 2020;11:1422–5.
59. Ahrén B, Landin-Olsson M, Jansson PA, Svensson M, Holmes D, Schweizer A. Inhibition of dipeptidyl peptidase-4 reduces glycemia, sustains insulin levels, and reduces glucagon levels in type 2 diabetes. J Clin Endocrinol Metab 2004;89:2078–84.
60. Deacon CF. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol 2019;10:80.
61. Mulvihill EE, Drucker DJ. Pharmacology, physiology, and mechanisms of action of dipeptidyl peptidase-4 inhibitors. Endocr Rev 2014;35: 992–1019.
62. Stoian AP, Sachinidis A, Stoica RA, Nikolic D, Patti AM, Rizvi AA. The efficacy and safety of dipeptidyl peptidase-4 inhibitors compared to other oral glucose-lowering medications in the treatment of type 2 diabetes. Metabolism 2020;109:154295.
63. Liu D, Pang J, Shao W, Gu J, Zeng Y, He HH, et al. Hepatic fibroblast growth factor 21 is involved in mediating functions of liraglutide in mice with dietary challenge. Hepatology 2021;74:2154–69.
64. Panjwani N, Mulvihill EE, Longuet C, Yusta B, Campbell JE, Brown TJ, et al. GLP-1 receptor activation indirectly reduces hepatic lipid accumulation but does not attenuate development of atherosclerosis in diabetic male ApoE(−/−) mice. Endocrinology 2013;154:127–39.
65. Baggio LL, Yusta B, Mulvihill EE, Cao X, Streutker CJ, Butany J, et al. GLP-1 receptor expression within the human heart. Endocrinology 2018;159:1570–84.
66. Zobel EH, Ripa RS, von Scholten BJ, Rotbain Curovic V, Kjaer A, Hansen TW, et al. Effect of liraglutide on expression of inflammatory genes in type 2 diabetes. Sci Rep 2021;11:18522.
67. Hadjiyanni I, Siminovitch KA, Danska JS, Drucker DJ. Glucagon-like peptide-1 receptor signalling selectively regulates murine lymphocyte proliferation and maintenance of peripheral regulatory T cells. Diabetologia 2010;53:730–40.
68. Sha S, Liu X, Zhao R, Qing L, He Q, Sun L, et al. Effects of glucagon-like peptide-1 analog liraglutide on the systemic inflammation in high-fat-diet-induced mice. Endocrine 2019;66:494–502.
69. Gupta NA, Mells J, Dunham RM, Grakoui A, Handy J, Saxena NK, et al. Glucagon-like peptide-1 receptor is present on human hepatocytes and has a direct role in decreasing hepatic steatosis in vitro by modulating elements of the insulin signaling pathway. Hepatology 2010;51:1584–92.
70. Boland ML, Laker RC, Mather K, Nawrocki A, Oldham S, Boland BB, et al. Resolution of NASH and hepatic fibrosis by the GLP-1R/GcgR dual-agonist Cotadutide via modulating mitochondrial function and lipogenesis. Nat Metab 2020;2:413–31.
71. Byun S, Seok S, Kim Y-C, Zhang Y, Yau P, Iwamori N, et al. Fasting-induced FGF21 signaling activates hepatic autophagy and lipid degradation via JMJD3 histone demethylase. Nat Commun 2020;11: 807.
72. Thorens B. Expression cloning of the pancreatic beta cell receptor for the gluco-incretin hormone glucagon-like peptide 1. Proc Natl Acad Sci U S A 1992;89:8641–5.
73. Dillon JS, Tanizawa Y, Wheeler MB, Leng XH, Ligon BB, Rabin DU, et al. Cloning and functional expression of the human glucagon-like peptide-1 (GLP-1) receptor. Endocrinology 1993;133:1907–10.
74. Campos RV, Lee YC, Drucker DJ. Divergent tissue-specific and developmental expression of receptors for glucagon and glucagon-like peptide-1 in the mouse. Endocrinology 1994;134:2156–64.
75. Wei Y, Mojsov S. Tissue-specific expression of the human receptor for glucagon-like peptide-I: brain, heart and pancreatic forms have the same deduced amino acid sequences. FEBS Lett 1995;358:219–24.
76. Bullock BP, Heller RS, Habener JF. Tissue distribution of messenger ribonucleic acid encoding the rat glucagon-like peptide-1 receptor. Endocrinology 1996;137:2968–78.
77. Pang J, Feng JN, Ling W, Jin T. The anti-inflammatory feature of glucagon-like peptide-1 and its based diabetes drugs-therapeutic potential exploration in lung injury. Acta Pharm Sin B 2022;12:4040–55.
78. Zhou W, Shao W, Zhang Y, Liu D, Liu M, Jin T. Glucagon-like peptide-1 receptor mediates the beneficial effect of liraglutide in an acute lung injury mouse model involving the thioredoxin-interacting protein. Am J Physiol Endocrinol Metab 2020;319:E568–78.
79. Toki S, Goleniewska K, Reiss S, Zhang J, Bloodworth MH, Stier MT, et al. Glucagon-like peptide 1 signaling inhibits allergen-induced lung IL-33 release and reduces group 2 innate lymphoid cell cytokine production in vivo. J Allergy Clin Immunol 2018;142:1515–28.e8.
80. Wei JP, Yang CL, Leng WH, Ding LL, Zhao GH. Use of GLP1RAs and occurrence of respiratory disorders: a meta-analysis of large randomized trials of GLP1RAs. Clin Respir J 2021;15:847–50.
81. Viby N-E, Isidor MS, Buggeskov KB, Poulsen SS, Hansen JB, Kissow H. Glucagon-like peptide-1 (GLP-1) reduces mortality and improves lung function in a model of experimental obstructive lung disease in female mice. Endocrinology 2013;154:4503–11.
82. Wong CK, McLean BA, Baggio LL, Koehler JA, Hammoud R, Rittig N, et al. Central glucagon-like peptide 1 receptor activation inhibits Toll-like receptor agonist-induced inflammation. Cell Metabol 2024;36: 130–43.e5.
83. Yokomori H, Ando W. Spatial expression of glucagon-like peptide 1 receptor and caveolin-1 in hepatocytes with macrovesicular steatosis in non-alcoholic steatohepatitis. BMJ Open Gastroenterol 2020;7: e000370.
84. Lee J, Hong SW, Rhee EJ, Lee WY. GLP-1 receptor agonist and non-alcoholic fatty liver disease. Diabetes Metab J 2012;36:262–7.
85. Pyke C, Heller RS, Kirk RK, Ørskov C, Reedtz-Runge 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.
86. Jensen CB, Pyke C, Rasch MG, Dahl AB, Knudsen LB, Secher A. Characterization of the glucagonlike peptide-1 receptor in male mouse brain using a novel antibody and in situ hybridization. Endocrinology 2018;159:665–75.
87. Liu D, Gu J, Shao W, Pang J, Qian X, Jin T. Comparison of beneficial metabolic effects of liraglutide and semaglutide in male C57BL/6J mice. Can J Diabetes 2022;46:216–24.e2.
88. Barra NG, Anhe FF, Schertzer JD. Immunometabolism sentinels: gut surface T-cells regulate GLP-1 availability. Endocrinology 2019;160: 1177–8.
89. Ussher JR, Drucker DJ. Cardiovascular actions of incretin-based therapies. Circ Res 2014;114:1788–803.
90. Hsieh J, Longuet C, Baker CL, Qin B, Federico LM, Drucker DJ, et al. The glucagon-like peptide 1 receptor is essential for postprandial lipoprotein synthesis and secretion in hamsters and mice. Diabetologia 2010;53:552–61.
91. Farr S, Taher J, Adeli K. Glucagon-like peptide-1 as a key regulator of lipid and lipoprotein metabolism in fasting and postprandial states. Cardiovasc Hematol Disord Drug Targets 2014;14:126–36.

92. Taher J, Baker CL, Cuizon C, Masoudpour H, Zhang R, Farr S, et al. GLP-1 receptor agonism ameliorates hepatic VLDL overproduction and de novo lipogenesis in insulin resistance. Mol Metabol 2014;3:823–33.
93. Liu J, Yang K, Yang J, Xiao W, Le Y, Yu F, et al. Liver-derived fibroblast growth factor 21 mediates effects of glucagon-like peptide-1 in attenuating hepatic glucose output. EBioMedicine 2019;41:73–84.
94. Le TDV, Fathi P, Watters AB, Ellis BJ, Besing G-LK, Bozadjieva-Kramer N, et al. Fibroblast growth factor-21 is required for weight loss induced by the glucagon-like peptide-1 receptor agonist liraglutide in male mice fed high carbohydrate diets. Mol Metabol 2023;72:101718.
95. Seghieri M, Rebelos E, Gastaldelli A, Astiarraga BD, Casolaro A, Barsotti E, et al. Direct effect of GLP-1 infusion on endogenous glucose production in humans. Diabetologia 2013;56:156–61.
96. Feng JN, Jin T. Hormones that are involved in metabolic homeostasis: overview of the past century and future perspectives. Obes Med 2022; 32:100422.
97. Badakhshi Y, Jin T. Current understanding and controversies on the clinical implications of fibroblast growth factor 21. Crit Rev Clin Lab Sci 2021;58:311–28.
98. Badman MK, Pissios P, Kennedy AR, Koukos G, Flier JS, Maratos-Flier E. Hepatic fibroblast growth factor 21 is regulated by PPARalpha and is a key mediator of hepatic lipid metabolism in ketotic states. Cell Metabol 2007;5:426–37.
99. Inagaki T, Dutchak P, Zhao G, Ding X, Gautron L, Parameswara V, et al. Endocrine regulation of the fasting response by PPARalpha-mediated induction of fibroblast growth factor 21. Cell Metabol 2007;5:415–25.
100. Kharitonenkov A, DiMarchi R. FGF21 revolutions: recent advances illuminating FGF21 biology and medicinal properties. Trends Endocrinol Metabol 2015;26:608–17.
101. Markan KR, Naber MC, Ameka MK, Anderegg MD, Mangelsdorf DJ, Kliewer SA, et al. Circulating FGF21 is liver derived and enhances glucose uptake during refeeding and overfeeding. Diabetes 2014;63: 4057–63.
102. Potthoff MJ, Kliewer SA, Mangelsdorf DJ. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. Gene Dev 2012;26:312–24.
103. Goetz R, Beenken A, Ibrahimi OA, Kalinina J, Olsen SK, Eliseenkova AV, et al. Molecular insights into the klotho-dependent, endocrine mode of action of fibroblast growth factor 19 subfamily members. Mol Cell Biol 2007;27:3417–28.
104. Kharitonenkov A, Shiyanova TL, Koester A, Ford AM, Micanovic R, Galbreath EJ, et al. FGF-21 as a novel metabolic regulator. J Clin Invest 2005;115:1627–35.
105. Kharitonenkov A. FGFs and metabolism. Curr Opin Pharmacol 2009;9: 805–10.
106. Vernia S, Cavanagh-Kyros J, Garcia-Haro L, Sabio G, Barrett T, Jung DY, et al. The PPARalpha-FGF21 hormone axis contributes to metabolic regulation by the hepatic JNK signaling pathway. Cell Metabol 2014; 20:512–25.
107. Lundasen T, Hunt MC, Nilsson LM, Sanyal S, Angelin B, Alexson SE, et al. PPARalpha is a key regulator of hepatic FGF21. Biochem Biophys Res Commun 2007;360:437–40.
108. Kharitonenkov A, Wroblewski VJ, Koester A, Chen YF, Clutinger CK, Tigno XT, et al. The metabolic state of diabetic monkeys is regulated by fibroblast growth factor-21. Endocrinology 2007;148:774–81.
109. Potthoff MJ, Inagaki T, Satapati S, Ding X, He T, Goetz R, et al. FGF21 induces PGC-1alpha and regulates carbohydrate and fatty acid metabolism during the adaptive starvation response. Proc Natl Acad Sci U S A 2009;106:10853–8.
110. Shao W, Jin T. Hepatic hormone FGF21 and its analogues in clinical trials. Chronic Dis Transl Med 2022;8:19–25.
111. Badman MK, Koester A, Flier JS, Kharitonenkov A, Maratos-Flier E. Fibroblast growth factor 21-deficient mice demonstrate impaired adaptation to ketosis. Endocrinology 2009;150:4931–40.
112. Dushay JR, Toschi E, Mitten EK, Fisher FM, Herman MA, Maratos-Flier E. Fructose ingestion acutely stimulates circulating FGF21 levels in humans. Mol Metabol 2015;4:51–7.
113. Fazeli PK, Lun M, Kim SM, Bredella MA, Wright S, Zhang Y, et al. FGF21 and the late adaptive response to starvation in humans. J Clin Invest 2015;125:4601–11.
114. Zeng K, Tian L, Patel R, Shao W, Song Z, Liu L, et al. Diet polyphenol curcumin stimulates hepatic Fgf21 production and restores its sensitivity in high-fat-diet-fed male mice. Endocrinology 2017;158:277–92.
115. Yang M, Zhang L, Wang C, Liu H, Boden G, Yang G, et al. Liraglutide increases FGF-21 activity and insulin sensitivity in high fat diet and adiponectin knockdown induced insulin resistance. PLoS One 2012;7: e48392.
116. Lee J, Hong SW, Park SE, Rhee EJ, Park CY, Oh KW, et al. Exendin-4 regulates lipid metabolism and fibroblast growth factor 21 in hepatic steatosis. Metabolism 2014;63:1041–8.
117. Nonogaki K, Hazama M, Satoh N. Liraglutide suppresses obesity and hyperglycemia associated with increases in hepatic fibroblast growth factor 21 production in KKAy mice. BioMed Res Int 2014;2014:751930.
118. 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.
119. Wester A, Shang Y, Toresson Grip E, Matthews AA, Hagstrom H. Glucagon-like peptide-1 receptor agonists and risk of major adverse liver outcomes in patients with chronic liver disease and type 2 diabetes. Gut 2024;73:835–43.
120. Fisher FM, Kleiner S, Douris N, Fox EC, Mepani RJ, Verdeguer F, et al. FGF21 regulates PGC-1alpha and browning of white adipose tissues in adaptive thermogenesis. Gene Dev 2012;26:271–81.
121. Lynch L, Hogan AE, Duquette D, Lester C, Banks A, LeClair K, et al. iNKT cells induce FGF21 for thermogenesis and are required for maximal weight loss in GLP1 therapy. Cell Metabol 2016;24:510–9.
122. Geng L, Feng Y, Li D, Nan N, Ma K, Tang X, et al. Meningoencephalitis, coronary artery and keratitis as an onset of brucellosis: a case report. BMC Infect Dis 2020;20:654.
123. Geng L, Lam KSL, Xu A. The therapeutic potential of FGF21 in metabolic diseases: from bench to clinic. Nat Rev Endocrinol 2020;16:654–67.
124. Gilroy CA, Capozzi ME, Varanko AK, Tong J, D'Alessio DA, Campbell JE, et al. Sustained release of a GLP-1 and FGF21 dual agonist from an injectable depot protects mice from obesity and hyperglycemia. Sci Adv 2020;6:eaaz9890.
125. Pan Q, Lin S, Li Y, Liu L, Li X, Gao X, et al. A novel GLP-1 and FGF21 dual agonist has therapeutic potential for diabetes and non-alcoholic steatohepatitis. EBioMedicine 2021;63:103202.