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Glucagon-Like Peptide-1: The Old, The New and The Unknown

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Abstract

Knowledge of the structure and action of glucagon-like peptide-1 (GLP-1) and its effects on inhibiting the enzyme responsible for its cleavage, dipeptidyl peptidase-4 (DPP-4), brought about great advances in the treatment type 2 diabetes mellitus (T2DM). However, both the effect of this incretin and the inhibition of the enzyme that promotes its inactivation have other benefits because the GLP-1 receptor (GLP-1R) is found in many different types of tissues of the human body. The objective of this review is to discuss old and new concepts on the physiology of this gut hormone, its pancreatic actions, and its different pleiotropic effects and consider possible new purposes for incretin-based therapy.

Key words

glucagon-like peptide-1; incretin; dipeptidyl peptidase-4; diabetes; obesity

Abbreviations

DPP4: dipeptidyl peptidase-4

DPP4i: dipeptidyl peptidase-4 inhibitor

GLP-1: glucagon-like peptide-1

GLP-1R: glucagon-like peptide-1 receptor

GLP-1Ar: glucagon-like peptide-1 receptor agonist

GLUT: glucose transporter type

GIP: gastric insulinotropic peptide

GRP: gastrin-releasing peptide

MUFA: monounsaturated fatty acid

RYGB: Roux-en-Y gastric bypass

SGLT1: sodium/glucose cotransporter type 1

T1DM: type 1 diabetes mellitus

T2DM: type 2 diabetes mellitus

Introduction

Glucagon-like peptide-1 (GLP-1) is a peptide synthesized from proglucagon, with homology in the amino acid sequence of glucagon itself. It is secreted by enteroendocrine L-cells located in the distal portions of the intestine (ileum and colon) [1,2]. Initially it is formed as an inactive molecule containing 37 amino acids that is later cleaved to form an active peptide of 30 amino acids, GLP-1 (7-36) [3]. The ingestion of food, especially carbohydrates and fat, and neurological stimuli are determining factors for the secretion of GLP-1[1,4]

In the bloodstream, GLP-1 [7-36] is broken down in GLP-1 [9-36] about 1-2 minutes by the enzyme dipeptidyl peptidase-4 [DPP-4], also known as CD26. This peptidase is found in all the circulation and is responsible for reducing active GLP-1 levels 5 (figure 1). To perform its different functions, GLP-1 binds to a membrane receptor (GLP-1R) linked to a G protein related to adenylate cyclase that was mapped in the 1990s on chromosome 6p21.1 [6,7].These receptors are expressed in several tissues including the pancreas, lung, gastrointestinal tract, heart, smooth muscle cells of blood vessels, endothelial cells, central nervous system, peripheral nervous system, kidneys, lymphocytes and macrophages[8,9].

Knowledge of the incretins in the pathophysiology of type 2 diabetes mellitus (T2DM) has been essential in the development of new therapies for this disease. Thus, the use of incretin mimetic drugs or incretin-based therapy - including GLP1 receptor agonists (GLP-1Ra) and DPP-4 inhibitors (DPP-4i) - started to be used in the treatment of T2DM to increase insulin secretion and reduce glucagon levels [1,8,10]. However,

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Figure 1: Intestinal production of GLP-1 and its cleavage by DPP-4
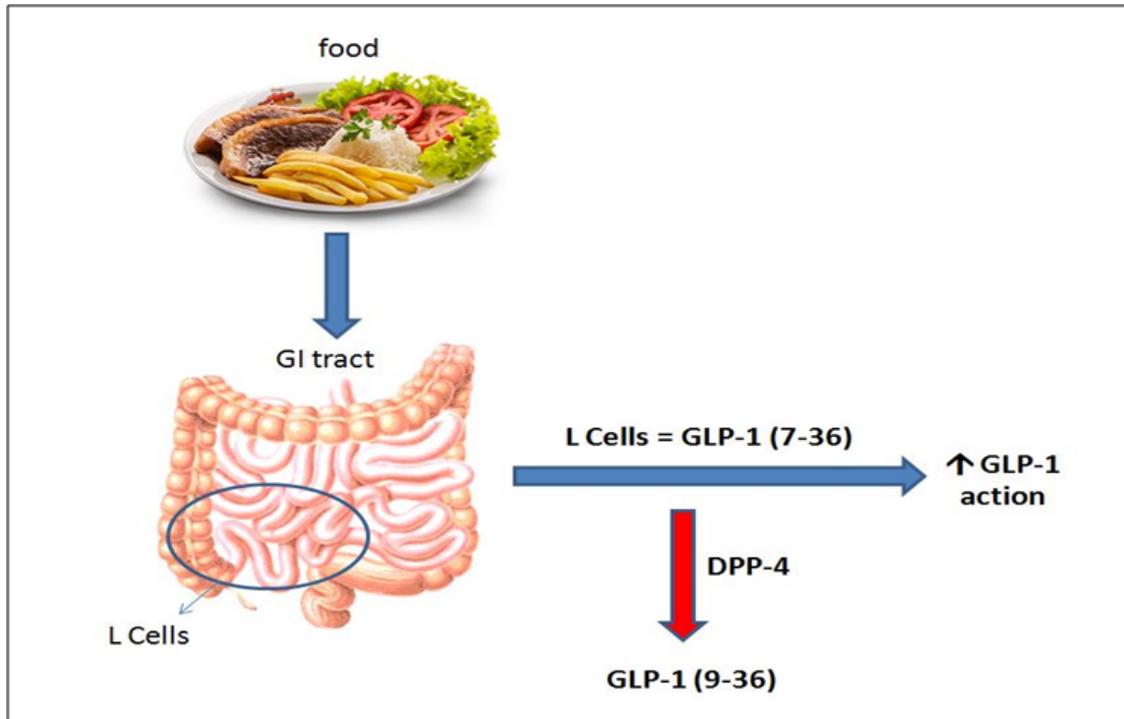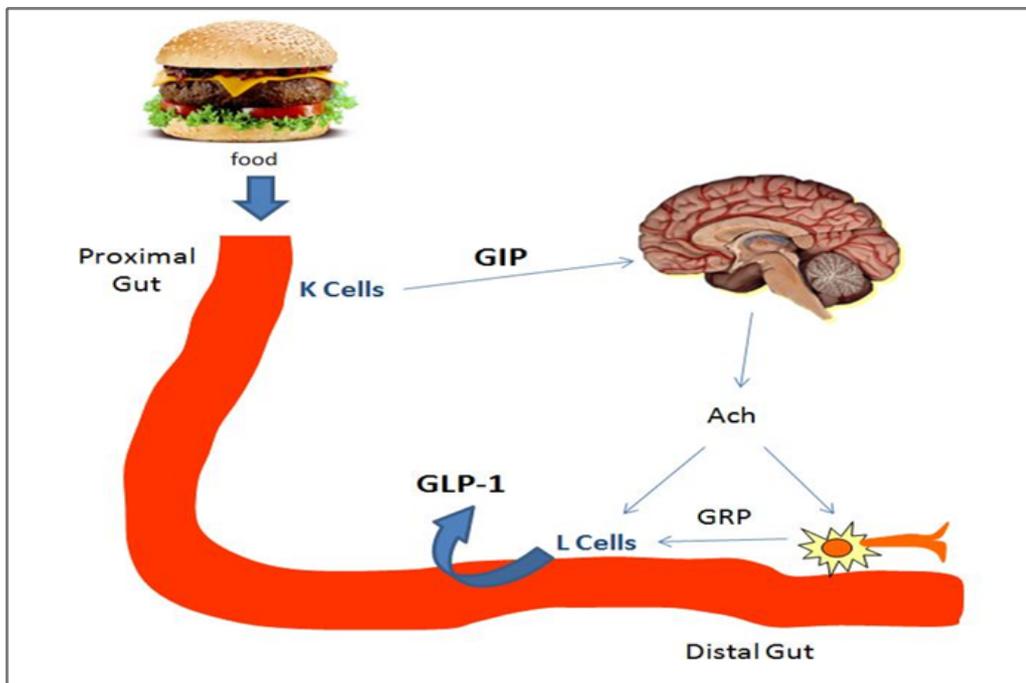

Figure 2: The relationship between K cells, L cells e neuropeptides

GIP: Gastric Insulinotropic Peptide
GLP1: Glucagon-like Peptide-1
Ach: Acetylcholine
GRP: Gastrin-releasing Polypeptide

because GLP-1R exists in different parts of the body, we are still learning about new and important pleiotropic effects that these medications can provide[9] . This review aims to examine old concepts and new findings on the effects of GLP-1 and discuss prospects for future applications of this therapeutic class.

L Cells: foods and neurotransmitter

In 1902, Starling and Bayliss hypothesized that the intestinal epithelium produces hormones that stimulate pancreatic secretions[11]. However, it was only 30 years later that these substances were named incretins by La Barre [12]. The importance of intestinal tract food on the secretion of GLP-1 is known since 1968 when Nauck et al. demonstrated a difference between oral ingestion of glucose and intravenous glucose and the reduced effect of incretins in patients with T2DM [13].

However, as GLP-1 is secreted in the more distal position in the gastrointestinal tract, there are doubts as to what kind of food would still arrive intact in that region to cause its release[14] . Even so, there is no doubt about the rapid increase of GLP-1 that is seen after bariatric surgery, in particular after the Roux-en-Y gastric bypass (RYGB) technique, when L cells adopt a more proximal position in the digestive process of the patient. This sudden increase in GLP-1 is one of the main explanations for the rapid and significant glycemic control in diabetic patients after bariatric surgery, even before they begin to lose weight[15] .

Glucose is an important trigger for L cells to secrete GLP-1 because of important flags on the cell surface including ATP-sensitive potassium channels, glucose transporter type 2 (GLUT2), glucokinase and sodium/glucose cotransporter type 1 (SGLT-1) [16-19]. Knowledge of this is important because of associations with other oral antidiabetic agents, such as metformin and alpha glucosidase inhibitors (in this case, miglitol), and their contribution to the secretion of GLP-1. Animal studies using a SGLT-1 inhibitor, phlorizin, caused a reduction in GLP1 secretion showing the importance of glucose absorption by L cells on the secretion of this incretin. There are no reports in the scientific literature, but the use of canagliflozin, a selective inhibitor of SGLT2 and SGLT1 used in the treatment of T2DM, could impair the release of GLP-1. Fat, particularly monounsaturated fat, also promotes increased GLP-1 levels because of the presence of long chain monounsaturated fatty acid (MUFA) transporters on the L cell surface [20].

Similar to insulin, the release of GLP-1 occurs in two phases: rapid and delayed [21]. Rapid secretion of this incretin occurs before the food arrives at the L cells and thus a theory of neurological involvement has been suggested, possibly related to the stimulation of K cells by food in the proximal intestine, which secrete gastric insulinotropic peptide (GIP), and hence involving the vagus nerve. M1 and M2 muscarinic receptors are involved in the release of GLP-1 [22-23]. This hypothesis was proven by comparing two groups of animals receiving stimulation of GLP-1 secretion (maltose plus miglitol). One

of the groups also received atropine and had a smaller increase in this gut hormone compared to those that did not receive this muscarinic antagonist that inhibits the parasympathetic nervous system [24] . A potent stimulator of this incretin is neural activity related to the neuropeptide, gastrin-releasing polypeptide (GRP)[25].Two recent articles from one research group show conflicting results regarding the possible stimulation of encapsulated glutamine in increasing GLP-1 release[26-27] . There are leptin receptors in the L cells of rodents and humans and leptin is able to stimulate the in vitro secretion of GLP-1, but this does not occur in rats whose obesity is induced by a high fat diet ant that are leptin resistant[28] . Although proteins are not related to the secretion of GLP-1, publications place whey proteins as potential enhancers of this incretin, but suggest that these results need further investigations [29].

The pancreatic effects of GLP-1

Among the incretins, GLP-1 stands out from the metabolic point of view, mainly due to its direct effects on pancreatic islets. GLP-1 promotes an increase in insulin biosynthesis and secretion, decreases in glucagon release, and both enhances proliferation and reduces apoptosis of β cells, the cells that release insulin [31-33]. This incretin binds to its receptor on the membrane of β cells and causes the stimulation of protein C resulting in increased cyclic adenosine monophosphate (cAMP) and protein kinase A (PKA) with consequent changes in ion channels. These changes include the inhibition of ATP-sensitive potassium channels, membrane depolarization, the opening of calcium channels with intracellular increases in calcium concentrations and insulin granule exocytosis [1]. The cAMP generated by the activation of GLP-1R may be responsible for the secretion of 70% of the total insulin [34].

The use of GLP-1 in animal models of type 1 diabetes (T1D) reduces inflammation in the remaining β cells and decreases their apoptosis, which is associated with the activation of the protein kinase C (PKC)/phosphatidylinositol 3-kinase (PI3K) and PKA/cAMP pathways[35] . The activation of the PKC/PI3K pathway by the GLP-1 in β cells promotes an increase in pancreatic duodenum homeobox 1 (PDX-1), an essential transcription factor for pancreas development and β cell function[36] . GLP-1 promotes an increase in somatostatin release, which seems to be a direct effect of GLP-1 on the presence of GLP-1R in the δ cells in islets of Langerhans [37]. The reduction in glucagon secretion from α cells occurs by mechanisms that are not fully understood. This effect could be indirectly related to insulin and/or somatostatin secretion via GLP-1[1] . Studies show a decrease in glucagon secretion due to GLP-1 in T1D patients with no residual insulin secretion [38-39].

In addition to direct effects on the pancreas, GLP-1 also has indirect metabolic effects by increasing glucose uptake in adipose and muscle tissues and decreasing hepatic glucose secretion, which, together with the reduction of appetite and decreased gastric emptying, contribute to better insulin sensitivity and

glycemic control[1] . For this reason, drugs in the GLP-1Ra and DPP-4i classes are of the utmost importance in the treatment of T2DM patients. Several incretin mimetic medications are used to treat T2DM.

Of the GLP-1Ra class there are:

- Exenatide

- Liraglutide

- Lixisenatide

- Dulaglutide

- Albiglutide

Of the DPP-4i class there are:

- Sitagliptin

- Vildagliptin

- Saxagliptin

- Linagliptin

- Alogliptin

The pleiotropic effects of GLP-1

GLP-1R exists in different organs of the human body apart from the pancreas. The use of incretin mimetics – GLP-1Ra and DPP-4i – that have been on the market for more than a decade, brought new perspectives on the extrapancreatic effects of GLP-1 and the possibility of using this molecule in other pathological conditions.

1- GLP-1 and heart

Studies on cardiovascular risk and the use of incretin mimetics increased expectations about the effects of GLP-1 on the heart. Prior to these results, in vitro studies in animals and humans pointed to a possible cardioprotective effect[40] . However, the results of research to evaluate cardiovascular risk such as the SAVOR (saxagliptin) [41], EXAMINE (alogliptin) [42] and ELIXA trials (lixisenatide)[43] showed a neutral effect in non-inferiority studies in relation to mortality from cardiovascular disease in T2DM patients.

In vitro activation of GLP-1R in cardiomyocytes promotes increased phosphorylation of protein kinase B (PKB or Akt) and extracellular signal regulated kinase (ERK) [44], important growth factors of this cell and important in the glucose metabolism. Activation also has anti-apoptotic effect[45] . Studies in animals showed improved glucose uptake and translocation of the glucose transporter type 4 (GLUT4) in the myocardium after the use of recombinant GLP-1 via 5'-AMP-activated protein kinase (AMPK) [46]. In endothelial cells, GLP-1 promotes a reduction of oxidative stress, by decreasing of plasminogen activator inhibitor-1 (PAI-I), vascular cell adhesion protein 1 (VCAM-1) and tumor necrosis factor-alpha (TNF-α), important aspects in the pathophysiology of atherosclerosis[47,48] .

The administration of GLP-1 in animals caused an increase in heart rate and systolic and diastolic blood pressures, independent of catecholamines[49]. In humans, this drug is known to increase the heart rate and blood pressure after the administration of incretin mimetic medications, but there is a tendency to decrease one hour after of its application [50]. It is known that a slight reduction in blood pressure has a significant impact on T2DM patients in terms of mortality from cardiovascular disease [51]. Saxagliptin causes a reduction in blood pressure and endothelial adhesion molecules in hypertensive mouse models [52]. A recent meta-analysis of phase III studies showed a reduction in systolic and diastolic blood pressures of around 4 mmHg with the use of saxagliptin in isolation or with other medications [53] . Similar results were also obtained with sitagliptin[54] , but not with alogliptin[55] . Studies on the chronic use of a GLP-1Ra, such as exenatide or liraglutide, point to a reduction in blood pressure compared to other drugs, such as insulin, perhaps due to weight loss and an improved lipid profile[56] . Studies on DPP-4i reported slight but significant reductions in systolic blood pressure, independent of weight loss[57] .

Inhibition of DPP-4 has a direct effect on the myocardium. The induction of acute myocardial infarction (AMI) in DPP-4 knockout mice (-/-) enhanced cardiac parameters and survival rates compared to wild animals with AMI 58. Wistar rats had a smaller ischemic area following the administration of vildagliptin[59] . The same applies to the use of linagliptin in animal models after ligation of the anterior descending coronary artery [60]. Other benefits of DPP-4 inhibition in conditions of improved reperfusion after ischemia are increases in stromal cell-derived factor-1 (SDF-1α) and progenitor cells in the region damaged with an increase in the expression of its receptor, CXCR4[57-61] . Clinical studies comparing granulocyte colony stimulating factor (G-CSF) or placebo combined with sitagliptin 100 mg/day for 28 days showed better regeneration of the myocardium with the synergistic use of a DPP-4i and G-CSF in respect to the migration of stem cells to the area of cardiac ischemia[62] .

The use of GLP-1 in rodents also showed an improvement in the lipid profile with a reduction in the absorption of triacylglycerol (TAG), decreases in its lymphatic transport, and lower production of the intestinal apolipoprotein B-48 (ApoB-48)[63] . The administration of sitagliptin in rats reduces TAG levels while fasting and after eating with this control occurring regardless of changes in gastric emptying[64] . A short treatment of daily injections of GLP-1 R was sufficient

to reverse hepatic steatosis in mice treated with a high fat diet[65]. The infusion of GLP-1 in healthy humans inhibited increases in TAG and free fatty acid levels after meals[9]. Other studies in humans with T2DM showed that the use of exenatide reduced postprandial TAG and ApoB-48 levels[66]. Moreover, there are indirect effects of GLP-1 on the heart such as improved insulin sensitivity of the myocardium and weight loss due to reduced appetite. However, the in vitro effects, results in animals and the possible theoretical benefits of GLP-1 on the heart have to be carefully analyzed [1,8].

Research shows that infusion of GLP-1 improved left ventricle ejection fraction and the hemodynamic parameters of the left ventricle in addition to increasing insulin sensitivity of the myocardium in dogs with induced heart failure[67]. Initial studies on the infusion of GLP-1Ra for six weeks in 12 patients with New York Heart Association functional class III/IV heart failure showed improvements in left ventricular function, oxygen consumption and in the six-minute walk test[68]. Inhibition of DPP-4, in theory, is beneficial to the heart as this enzyme, as well as increasing GLP-1 levels, promotes cardioprotective changes by other mechanisms. These mechanisms include increasing GIP (increased lipogenesis), SDF-1α (recruitment of progenitor cells to areas of ischemia and angiogenesis), neuropeptide Y (NPY - increased lipogenesis and angiogenesis) and peptide YY (PYY - increased collateral circulation and reduced lipolysis) [40-57]. Nevertheless, results of the SAVOR trial showed an increase in the number of hospitalizations for heart failure[41], results that are still disputed and not reported by other studies using incretin mimetic drugs. Studies on the use of sitagliptin in db/db mice did not report any improvement in systolic function but showed reduced myocardial fibrosis and improvements in the degree of left ventricular relaxation, indicating a possible benefit in diastolic function[69]. Isolated studies and meta-analyses of results using saxagliptin showed that this group of drugs in patients with heart failure caused significant differences in diabetic and non-diabetic individuals[57]. Human studies on the infusion of GLP-1 after AMI and angioplasty showed overall improvement in left ventricular function and reductions in intra-hospital mortality and length of hospital stay[1,56]. While some studies point to an improvement in the ejection fraction, there were no significant changes in the amounts of brain-derived natriuretic peptide (BNP), a substance produced in the heart ventricles that is essential for natriuresis, vasodilation, and suppression of adrenergic activity and a marker of acute heart failure[70,71].

Furthermore, GLP-1 has a well-known and important endothelial protective role in reducing oxidative stress in the vessel wall. Studies with alogliptin suggest that GLP-1 has an independent vasodilator effect[72]. Any DPP-4i, such as GLP-1Ra, acts on the endothelium increasing phosphorylation of Akt and endothelial nitric oxide synthesis (eNOS) and thus increases nitric oxide (NO) production[56,57]. In a study using obese Zucker rats, the administration of saxagliptin promoted NO

synthesis before the hypoglycemic effect of the medication[73]. Inhibition of DPP-4 increased the collagen content of atherosclerotic plaque and reduced the migration of monocytes in response to monocyte chemotactic protein-1 (MCP-1)[74]. This vasodilator effect was also shown in humans with T2DM with an injection of acetylcholine after the administration of vildagliptin giving a significant increase in vasodilation compared to a group receiving acarbose[75]. A study on T2DM that used animal models also showed a decrease of advanced glycation end-products (AGEs), their receptors (RAGEs) and oxidative stress markers such as intercellular adhesion molecule 1 (ICAM), transforming growth factor-beta (TGF-β) and PAI-I, after the administration of vildagliptin and sitagliptin[76-78]. More recent studies in rats indicate the presence of GLP-1R only in the atrium and not in the ventricles, and this has an important impact on the beat and heart actions in patients submitted to incretin-based therapy[79].

2- GLP-1 and the nervous system

The best-known pleiotropic effects of GLP-1 on the nervous system is the reduction in appetite with resulting weight loss[80]. Intraventricular injections of GLP-1 in rat brains show that the higher the concentration of this incretin in the brain the lower the food consumption is[81]. Apart from glycemic control and the therapeutic indication for T2DM patients, studies show that higher doses of liraglutide are effective in reducing weight in non-diabetic obese patients. In 2015, after approval by the FDA, liraglutide (3.0 mg) was launched in the United States for the treatment of obesity [82,83].

This effect could be explained by several effects of GLP-1 on the central and peripheral nervous systems as well as by its direct influence on taste receptors[84] and its hypothalamic action with increases in the action of anorectic neurons[85]. Incretins have a key role in the entero-insular axis to control the appetite and metabolism[1]. The use of GLP-1Ra is associated with side effects such as nausea and vomiting because of reduced gastric emptying, and this contributes to the indirect anorectic effect of this medication[9].

In spite of the effects on the central nervous and enteric systems (see below in section 7), there are very few reports in the scientific literature about the effect of incretin mimetic medications in relation to the peripheral nervous system or possible effects on peripheral neuropathy. In vitro studies show an anti-apoptotic effect of exendin-4 in cultured rat hippocampal neurons, with a potential neuroprotective effect in animal models of sensory neuropathy; the central infusion of GLP-1 in rats reduced the levels of the β-amyloid peptide[86].

3- GLP-1 and the lung

Despite being one of the first places where the presence of GLP-1R was identified in the human body [87], there are few studies on the effects of incretin on the lung. Some in vitro research and animal studies have shown a vasodilator

effect of GLP-1 on the pulmonary artery related to NO[88] . After case reports of rapid improvements in pulmonary arterial hypertension in obese T2DM patients after bariatric surgery[89] , our group published a report of two cases of T2DM patients with idiopathic pulmonary arterial hypertension who presented reductions in diastolic blood pressure of the right ventricle without weight loss after six months of vildagliptin use (50 mg b.i.d.)[90] . This effect was credited to an increase in GLP-1 levels but more studies are needed to confirm this.

Other studies show additional pleiotropic effects of GLP-1 in the lung in animal models such as increased surfactant production by type II pneumocytes[91] and an anti-inflammatory effect in animal models of acute respiratory failure[92] . The action of GLP-1 on the lungs requires further, more consistent studies to validate these potential pleiotropic effects in humans.

4- GLP-1 and the Kidney

The effect of GLP-1 on the kidneys is not completely understood. Studies on the infusion of GLP-1 in healthy adults showed a significant reduction in sodium reabsorption in the proximal tubules and a possible reduction in the angiotensin II production[93] . Inhibition of DPP-4, in addition to increasing native GLP-1 levels also appears to contribute to a decrease in the activity of the renin-angiotensin system. A study using vildagliptin in animals showed diminished renal damage due to a reduction in the production of transforming growth factor-β1 (TGF-β1)[94] . A similar study with linagliptin improved the renal oxidative stress and reduced albuminuria levels [95].

Studies with animal models of T1D reported reduced renal failure after infusions of liraglutide.[96] Apart from the effect on the renin-angiotensin system, the use of GLP-1 showed a better effect on eNOS activity by inhibiting the nuclear factor-kappa B (NF-kB) pathway. A study on alogliptin in T2DM patients with microalbuminuria described an improvement in renal function with reduced renal stress on using DPP-4i in the early stages of diabetic nephropathy to activate the SDF-1α/cAMP pathway[97] .

5- GLP-1 and the liver

The liver plays a key role in the pathophysiology of T2DM with the production of hepatic glucose due to insulin resistance and the increase in glucagon[98]. As most T2DM patients have risk factors for metabolic syndrome, a large number of patients may have hepatic steatosis or non-alcoholic fatty liver disease (NAFLD), which can lead to severe liver complications such as non-alcohol steatohepatitis (NASH) and even cirrhosis[99] .

GLP-1 has a fundamental role in respect to the islets of Langerhans, not only in the insulin-producing β cells, but also in α cells which help to reduce glucagon secretion and thus a reduction in gluconeogenesis and glycogenolysis, leading to improved glycemic control[1] . In addition to the weight loss associated with the increase of GLP-1[83], studies describe an improvement in insulin sensitivity of hepatocytes because of

increased phosphorylation of AMPK and reversion of hepatic steatosis due to the stimulation of fatty acid oxidation [100,101]. A study with liraglutide demonstrated reduced insulin resistance and decreased lipotoxicity in patients with NASH [102]. Human studies that used exenatide, liraglutide and sitagliptin point to a significant improvement in hepatic steatosis, independent of weight loss, and indicate incretin mimetic drugs as a therapeutic option for this comorbidity [103].

6- GLP-1 and the bone

Patients with T2DM exhibit increased risk of bone fractures when compared with non-diabetic patients [104]. Some oral hypoglycemic agents, especially the insulin sensitizer pioglitazone, increase the risk of developing osteoporosis and fractures[105] . Previous studies have shown a neutral effect on bone mass using DPP-4i. Research points to a beneficial effect of GIP and glucagon-like peptide-2 (GLP-2) on the bone, but the effects of GLP-1 are not clear yet[106] . GLP-1R knockout mice have osteopenia and cortical bone fragility and this suggests a possible action of GLP-1 on osteoblasts[107] . osteoporosis animal models induced by ovariectomy improved with the use of exendin-4, which prevents deterioration of the bone trabecular structure, increases bone formation markers (ProPeptide of type 1 procollagen - P1NP) and decreases bone resorption markers (C- terminal telopeptide of type 1 collagen - CTX1)[108] .

It is known that weight loss, one of the pleiotropic effects of GLP-1, may also contribute to bone fragility[109] . A recent study in obese non-diabetic postmenopausal women taking liraglutide showed increased bone formation (elevation of P1NP without changes in CTX1) with prevention of bone loss even after weight loss due to the medication[110] . Studies in diabetic insulin-resistant rats show involvement of osteogenic activation of the Wnt pathway that can be reversed using exendin-4 [111].

7- GLP-1 and the gastrointestinal tract

The effect of GLP-1 of delayed gastric emptying related to GLP-1R in gastric parietal cells is well known[1,112] . It is believed that GLP-1 has direct effects on gastric emptying and the inhibition of gastric acid secretion and indirect effects associated with the parasympathetic innervation of the body[113].

The mechanisms involved in the inhibition of gastrointestinal motility are unclear but appear to be associated with vagal afferent fibers[114,115] . Recently, GLP-1R was identified in the intestine (small and large) and in enteric neurons of several animal species, including humans[79,115] . Recent in vitro studies identified the presence of GLP-1R in muscle cells of the human colon with exposure to GLP-1 reducing the amplitude of spontaneous contractions due to the neural production of NO but without altering the frequency and basal tone of contractions at rest. This action could contribute to delaying enteric propulsion, increasing the absorption of water

and electrolytes and may cause constipation[116] .

We know that epigastric pain and diarrhea are common symptoms in patients taking metformin [117]. The reduced secretion of acid by the stomach and decreased colon motility due to the action of GLP-1 on its receptor in these organs[113], could explain the reduced presence of side effects related to metformin in patients who are taking this drug in combination with a PPD-4i.

8- GLP-1 and blood cells

As mentioned above, an important effect of the inhibition of DPP-4 is an increase in SDF-1α [61]. This facilitates the homing of bone marrow progenitor cells to areas of damage and their binding to their receptor CXCR4, thereby helping the tissue restoration process. Evidence points to a positive influence on macrophage-mediated inflammatory response in an in vitro study with alogliptin; this could contribute to tissue remodeling and wound healing [118].

The breakthrough in the treatment of HIV, the increased survival and adverse effects of antiretroviral therapy are associated with the emergence of problems related to metabolic syndrome and cardiovascular diseases, including diabetes[119]. The use of incretin mimetic medications is an interesting therapeutic option since it is known that the activity of the DPP-4 enzyme is altered in diabetic patients with HIV. DPP-4 is a peptidase similar to the CD26 surface protein and so it is involved in signaling and interactions between antigen-presenting cells and CD4+ T lymphocytes[120] . It was not known whether inhibition of DPP-4 in HIV patients would be beneficial or dangerous. Adverse effects in respect to the immune system, the viral load or in connection with activation of the immune system were not noticed in a recent study of HIV-positive non-diabetic patients who received sitagliptin, and so it appears that this drug class is safe in diabetic patients at high cardiovascular risk [121].

9- GLP-1 and adipose tissue

Adipose tissue plays a crucial role in the pathophysiology of T2DM and glycemic control, and GLP-1 promotes better insulin sensitivity in this tissue[122] . In vitro GLP-1 promotes an increase of lipolysis in adipocytes of mice[123] and has lipogenic and lipolytic effects on human adipocytes[124]. Despite the incretin effects on adipose tissue, the presence of GLP-1R is uncertain at this location. At this time, despite being broken down by the action of adrenergic fibers in this tissue, neuropeptide Y (NPY) can be affected by the action of DPP-4 and modulate the proliferation and differentiation of pre-adipocytes [125]. Animal studies with vildagliptin showed suppression of adipogenesis without affecting lipolysis and an ability to suppress peroxisome proliferator-activated receptor gamma (PPAR-γ) activity and stop the conversion of pre-adipocytes into mature adipocytes[126] . These findings are important keys to elucidate the glycemic control promoted by incretins and the

inhibition of DPP-4, as well as how they assist in weight loss, improve the lipid profile and increase the cardioprotective effect.

10- New discoveries about GLP-1

In addition to GLP-1Ra and DPP-4i, the increase in GLP-1 can be promoted by the use of other oral hypoglycemic medications used in the treatment of T2DM, such as metformin[127,128] and α-glucosidase inhibitors, especially miglitol[24] . Another way to promote an increase in GLP-1 is by bariatric surgery, especially when an intestinal bypass technique is used transferring L cells to a more proximal portion in the tract[129] . Studies after bariatric surgery clarified many doubts about incretins. A relatively common complication in the postoperative follow-up of these patients are episodes of hyperinsulinemic hypoglycemia[130] . Some studies have questioned the role of pre-surgical nesidioblastosis of β cells because of obesity and pre-surgical insulin resistance, as the main triggering factor of this complication[131,132] . In addition to nutritional interventions, other types of medical therapy exist for hypoglycemia such as octreotide, diazoxide and calcium channel blockers, especially nifedipine, and partial pancreatectomy[133]. However, the use of these therapies often have side effects and have little effectiveness to avoid hypoglycemic episodes.

We know that an improvement in glycemic control of obese T2DM patients after bariatric surgery occurs in the first weeks, even before the patient experiences any significant weight loss. In these cases, many patients are discharged without the need to continue using oral hypoglycemic agents. The main reason is the increase in GLP-1, which promotes insulinotropic effects in the pancreas[134]. Another factor that contributes greatly to the enhancement of this intestinal hormone after bariatric surgery is the change in dietary patterns, in most patients, with a higher percentage of fatty foods consumed, which stimulate the secretion of GLP-1 by L cells[135] . Recent studies point to this increase in GLP-1 as the main trigger of hyperinsulinemic hypoglycemia in this group of patients with a significant improvement in β-cell function and glucose tolerance evaluated using the mixed meal test after RYGB [136]. In the same study, the patients who received exendin 9-39, an antagonist of GLP-1R, had decreased sensitivity to glucose of β cells to pre-operative levels, increased secretion of glucagon and glucose intolerance. For this reason, an editorial published in 2014 entitled "Hypoglycemia after gastric bypass: the dark side of GLP-1" puts the GLP-1 blocker as a viable option for patients in this pathological situation[137] . However, it questions genetic variations such as personal predisposition to post-receptor response, as there is no difference in the GLP-1R density in pancreatic β cells of patients with asymptomatic hypoglycemia after RYGB[138]. Recent studies point to lower secretion of GLP-1 and GIP associated with weight gained after RYGB[139] . This result puts GLP-1Ra as a viable therapeutic option to treat weight gain following bariatric surgery.

Recent studies show the effect of bile acid sequestrants

on GLP-1. Medications used in the treatment of dyslipidemia that sequester bile acids have an important effect on the metabolism and on glycemic control in T2DM patients, and today these drugs are considered a therapeutic option for this disease[140,142]. In addition to the effects on bile acids, these medications change intestinal transit, improve the composition of the gut microbiota and increase the secretion of GLP-1 in L cells which also has an important implication in glycemic control in T2DM patients that use this type of medication[143] . In the clinical practice, questions remain about the beneficial metabolic effects of natural products as well as other possible stimulants of GLP-1. Studies have shown the effect of Panax ginseng, in particular, its most abundant constituent, ginsenoside Rb1 (Rb1) in models of diabetic rats[144] . Rb1 stimulates the secretion of GLP-1 which was reversed after the use of diazoxide and nifedipine[145]. As discussed above, whey protein, and encapsulated glutamine may stimulate the secretion of GLP-1 but more studies are needed to confirm this[29,146] . Studies have shown the presence of GLP-1R in the rat pituitary gland and the secretion of thyroid-stimulating hormone (TSH) in rat thyrotroph cultures, as well as an increase in the in vitro secretion of luteinizing hormone (LH), adrenocorticotropic hormone (ACTH), and vasopressin[147]. However, studies in humans with short-term infusions of GLP-1Ra showed only transient increases in circulating levels of ACTH and cortisol[148] . Recent studies demonstrated the effects of exendin-4 on the hypothalamic-pituitary-adrenal axis with secretion of corticosteroids in male mice[149] and in the reproduction of female mice by increases in LH and the number of mature follicles[150]. Despite the presence of GLP-1R in culture of parafollicular C-cell tumors, the use of GLP-1Ra was not associated with the diagnosis of medullary thyroid carcinoma in humans[83,151]

Since the beginning of incretin-based therapy in T2DM patients, questions remain about the use of this therapy in T1D patients[152] . Early use of this hormone could protect the remaining β cells and increase residual insulin secretion, as well as provide other benefits such as reducing the production of glucagon by α cells [38,153,154]. A study of nine patients with long-standing T1D, using high doses of insulin and with peptide C undetectable, after receiving treatment with liraglutide for a short period, achieved reductions in the mean blood glucose level, less variability in blood sugar levels and reductions in insulin dose without the risk of hypoglycemia [155]. A recent study detected the presence of GLP-1R in acinar cells of the exocrine pancreas and activation of cAMP appears to induce the secretion of amylase[156] .

Until recently, it was believed that adult humans no longer had brown adipose tissue. A recent study using F18-fluorodeoxyglucose positron-emission tomography-computed tomography (PET-CT) showed this type of tissue in adults, its importance in thermogenesis and its role in the pathophysiology of obesity[1587] . The same study also showed an inverse relationship between the thermogenic activity of this tissue

and the ambient temperature. Hibernomas, rare benign tumors that mimic the brown adipose tissue, cause significant weight loss[158] . Recent research shows a 'browning' process of adipocytes in white adipose tissue derived from the stem cells of the mesoderm, with differentiation into 'beige' adipocytes [159]. Different substances are implicated as possible promoters of this process, including GLP-1[160] . Despite an increase in thermogenesis of this tissue, the use of liraglutide in a model of diet-induced obese mice had little repercussion on the weight loss of animals [161].

Conclusions

Since the beginning of last century, the role of gut hormones and their effects on the pancreas and blood glucose control has been postulated. Over some 30 years, studies have brought us a better understanding of GLP-1 and for nearly one decade, we have been using incretin-based therapy (GLP-1Ra or DPP4-i) for glycemic control in T2DM patients, but we are still learning about the physiology of GLP-1 and its pancreatic and extra-pancreatic actions. Despite the results regarding the cardiovascular safety of these medications, we know their effectiveness in controlling blood glucose and their potential benefit in cardioprotection. Further studies are being carried out of other possible therapeutic benefits related to incretin mimetic drugs beyond diabetes and reduced cardiovascular risk.

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