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BACKGROUND.

The concept of the modulatory effect of intestinal peptides upon the function of the endocrine pancreas started early in 1906, when Starling and Bayliss proposed that the duodenum could release a chemical agent capable to enhance the "internal secretion" of the pancreas (1). Almost at that time, Moore tested the possible effect of the oral administration to diabetic patients of an acidic extract from intestinal mucosa upon the disease progression (2). Later, in 1932, the term "incretin" was coined to describe the effect of a gut hormone upon meal-stimulated insulin secretion (3,4).

Currently, it is well accepted that an oral glucose load elicits a higher release of insulin than that induced by an equivalent concentration of glucose obtained after its intravenous injection. This enhancing phenomenon, known as "incretin effect", has been ascribed to the release of enteropeptides (incretins) that are responsible for more than 50% of the amount of insulin released in response to glucose ingestion (5). Thus, by definition, incretins are insulinotropic peptides that control β-cell sensitivity to glucose.

Two peptides are currently considered the most representative and physiologically relevant incretins: glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic peptide (GIP). While GIP was isolated and sequenced in 1971, GLP-1 was first described in 1981 (4). Both peptides stimulate glucose-induced insulin secretion, reduce postprandial glycemia, and induce an increase of the β-cell mass in intact animal models; the latter effect results from a combination of increased β-cell proliferation and neogenesis rates, and decreased rate of apoptosis (6,7).

Even when many of these actions are shared by both peptides some other effects are not: GLP-1, but not GIP, slows down gastric emptying and reduces glucagon secretion as well as appetite (8). Additionally, exogenous infusion of GLP-1 promotes satiety and reduction in food intake both in animal and human models (9).

People with type 2 diabetes (T2DM) show a decreased incretin effect upon insulin secretion that in some cases can be due to decreased levels of GLP-1. However, most authors agree that such effect is mainly due to a decreased β-cell sensitivity to the peptide (4). On the other hand, decreased GIP action in T2DM is mainly associated to a decreased target tissue response (GIP resistance).

Caloric intake stimulates incretin release in a dose-dependent manner (10); the enhancing effect of incretins upon insulin secretion is "glucose dependent": the strong potentiation of exogenous administration of incretins upon glucose-induced insulin release is
only seen while glucose concentration is above fasting levels, and this effect decreases as the glucose concentration returns to normal fasting values; for this reason, its administration to treat diabetes decreases the risk of hypoglycemia (11).

Both, GLP-1 and GIP have a very short half life (1-2 minutes) because they are rapidly degraded in vivo by the activity of a post-alanine/post-proline peptidase (dipeptidyl peptidase 4, [DPP-4]) (12). Consequently, treatment of people with T2DM with native GLP-1 and GIP is limited because it requires their continuous subcutaneous infusion. Two main strategies have been designed to overcome this problem and to make incretins available for their use in daily T2DM treatment:

1. Development of GLP-1 analogues resistant to the DPP-4 degradation, such as exenatide and liraglutide; these two peptides must be administered by subcutaneous injection.
2. Development of specific DPP-4 inhibitors such as sitagliptin and vildagliptin, that can be administered orally (13).

PHYSIOLOGICAL AND METABOLIC EFFECTS OF ENDOGENOUS INCRETINS.

GLP-1 is produced and secreted by L cells from the distal ileum and colon (14). These cells contain proglucagon that after being processed by preprotein convertases (PC1 and PC2) produces GLP-1 and other bioactive peptides. The lack of expression of these two convertases is accompanied by a deficit in GLP-1 production, severe obesity, a deficit in intestinal absorption of sugars and other nutrients, hypoglycemia as well as other hormonal and metabolic disorders (15,16). The proglucagon gene also encodes GLP-2, which has no insulinotropic effect but displays some other interesting physiological activities.

While in humans more than two thirds of GLP-1 circulates as a COOH-terminally amidated form (GLP-1 [7–36] amide), it is also possible to detect a glycine extended form, known as GLP-1 (7–37) (4).

L cells release GLP-1 in response to nutrients, especially fats and carbohydrates (14,16), in a biphasic manner. The first phase is regulated in a complex way: GIP secreted by intestinal K-cells would stimulate acetylcholine release in celiac plexus terminals (17), which stimulate type M1 muscarinic receptors (18). Atropin (non-selective antagonist of muscarinic receptors) reduces GLP-1 response to either oral glucose load or food intake (19), showing the importance of the vagus nerve to mediate its secretion. The second phase of secretion is triggered by a direct effect of nutrients upon intestinal L-cells. The control of GLP-1 secretion is completed with a self-regulating negative feedback mechanism (20).

Once GLP-1 is released, it is degraded by the action of dipeptidyl-dipeptidase 4 (DPP-4) (21,22), which would also degrade other peptides, such as hypophyseal peptide (adenylate-cyclase activator), bradykinin and GIP (23). The administration of DPP-4 inhibitors to people with T2DM increases GLP-1/GIP levels and decreases significantly the amplitude of postprandial glycemic oscillations and the HbA1c levels (24).

GLP-1 binds to specific receptors located at the islet cells but also to different tissues such as stomach, CNS, heart (endocardium and myocardium), endothelial, bone and kidney tubular cells. These receptors are quite similar in humans and rats (90% homology); both consist in 463 aminoacid proteins, differing in only 42 positions. In pancreatic β-cells, GLP-1 couples to a specific G-protein; thus, GLP-1 binding to its specific receptors triggers a cascade that involves adenylate cyclase activation, increased adenosine-3',5'-cyclic monophosphate (cAMP) concentration (25), and
protein kinase A (PKA) activation (26). Through this pathway, GLP-1 promotes the phosphorylation of GLUT2, KATP and Ca\(^{2+}\) channels (26-28). cAPM would also activate a PKA-independent cascade: it interacts with GEF2 or Epac2 (cAMP sensor) forming a complex with Rim2 and activating Rab3 (a component of the exocytotic cell machinery) (29).

GLP-1 stimulates insulin and inhibits glucagon secretion by interacting with its islet β- and α-cell receptors (30). The inhibitory effect on glucagon secretion is maintained even in people with diabetes (31). Additionally, GLP-1 reduces the released pro-insulin/insulin ratio, indicating that β cells work at a lower and more efficient rate, thus decreasing its stress.

The insulinotropic effect of GLP-1 has also extrainsular components: GLP-1 acts as an hepatic portal glucose "sensor" which is activated whenever a glucose gradient is established between portal and peripheral blood, as it occurs after food intake. Such hepatic and portal sensor is the first contact with the ingested glucose, and would modulate insulin secretion through a neurohumoral pathway. This concept is supported by the existence of neurons from the enteric nervous system located in the pancreas which express K\(_{ATP}\) channels that would send signals when they get in contact with glucose (32). The glucose portal sensor promotes first-phase of insulin secretion, which is absent in double GLP-1 and GIP receptors KO mice (DIRKO mice) (32). These mice have also a marked decrease of the second-phase of insulin secretion. Such insulinotropic effect is responsible for more than 50% of the insulin secretion elicited by glucose intake, and disappears progressively during the development of T2DM due to the decrease of GLP-1 and GIP production/β-cell sensitivity particularly to GIP (33).

The importance of GLP-1 in glycemic homeostasis was demonstrated using mice with KO of GLP-1 receptors: they did not develop severe diabetes, but had impaired insulin secretion during the oral glucose tolerance test (OGTT) (32). On the other hand, GLP-1 secretion after food intake decreased in some people with T2DM diabetes (34).

Chronic administration of GLP-1 to rodents activates the transcription of genes involved in β-cell differentiation and function and in islet neogenesis such as Pdx-1, Glut2, glucokinase and insulin (6,7). The increase in β-cell mass is the consequence of a simultaneous increase in β-cell replication and islet neogenesis and a significant decrease in β-cell apoptotic rate. This latter effect was also observed in normal human islets cultured with exendin-4 (35).

GLP-1 perfusion normalizes fasting glycemia in people with T2DM (36) and decreases glycemic fluctuations after food intake (20,21). These effects are due to the combined effect of enhanced insulin and decreased glucagon secretion as well as hepatic glucose production and gastric emptying.

The effect of a single GLP-1 injection is short due to its rapid degradation, thus preventing its use in the treatment of T2DM. GLP-1 analogues such as exendin-4 and liraglutide administered to people with T2DM reduce significantly HbA1c levels, representing a valid alternative for the treatment of this type of diabetes (37).

**Extrapancreatic effects of GLP-1.**

GLP-1 affects other organs such as the central and autonomic nervous system, hypothalamus-pituitary axis, gastrointestinal tract and gastric emptying, muscle, adipose tissue, liver, bone, myocardium and cardiovascular system.

Injected into the CNS, GLP-1 and other GLP-1 receptor agonists inhibit food intake and weight gain in mice (14), while GLP-1 antagonists (e.g. exendin 9-39) inhibit
these effects. Both in humans and in experimental animals, GLP1 receptors are detectable at the nodose gangle neurons (vagal system) and hypothalamus. In animal models, effects of GLP-1 upon appetite, feeding and body weight are suppressed by subdiaphragmatic truncal vagotomy or the use of capsaicin, a well-known neurotoxic agent. Again, CNS actions of GLP-1 on appetite and body weight could be produced by both direct and indirect mechanisms (4).

GLP-1 exerts several effects upon the nervous system: it protects and reverses excitotoxic neuronal damage (38), decreases amyloid deposits and protects hippocampal neurons from amyloid-induced death (39), and protects neuronal cells from death induced by nerve growth factor deprivation (40). Exenatide stimulates neurogenesis, reverses key deficits (41) and induces recovery in rat Parkinson models (42). GLP-1 preserves primary cortical and dopaminergic neurons in cellular and rodent models of stroke and Parkinson disease (43).

Figure 1. Figure summarizes the multiple effects of GLP-1 and GIP on different tissues and organs. It can be seen that despite these two peptides are very important modulators of islet cell mass and function, their effect is far from being restricted to this microorgan. They really have a wider spectrum of action and are important components of the whole body organ/tissue network of signals than control the caloric/metabolic homeostasis as well as the regulation of many other important processes. It is therefore reasonable to expect that GLP-1/GIP dysfunction can trigger multiple alterations in body functions with pleiotropic clinical and metabolic manifestations. The highly active research devoted to the study of these peptides will certainly improve our knowledge and also allow to identify several alterations that can be selectively treated with their therapeutic replacement.
GLP-1 displays inhibitory effects on gastric acid secretion and motility, and it would mediate the "ileal break effect". It also slows down gastric emptying in human beings as well as in rodents, a relevant difference with respect to GIP; this effect is probably mediated by the nervous system (14). Vagal afferent denervation abolishes the deacceleration of gastric emptying induced by central administration of GLP-1. Although GLP-1 receptors are present at stomach and promote emptying rate reduction when activated, their action mechanism awaits for clarification.

In skeletal muscle strips, GLP-1 and exenatide seem to stimulate the activity of glycogen synthase α and of glycogen synthesis. These effects would be linked to an activation of PI3K/PKB and p42/44 MAPK pathways. The activation of PI-3K/PKB is probably the mediator for the increased glucose uptake induced in muscle cells by GLP-1 receptor agonists (4).

As mentioned before, GLP-1 displays important effects on myocardium and the cardiovascular system. There are GLP-1 receptors in the heart (44) and on the vascular endothelium (45). Mice with a GLP-1 receptor knockout have impaired left ventricle contractility and diastolic functions (46). Conversely, GLP-1 protects rat myocardium against subsequent ischaemia/reperfusion injury (47) and improves cardiac function in dogs with chronic heart failure (48). Perfusion of GLP-1 can also improve left ventricular ejection fraction in people with chronic heart failure or with left ventricular dysfunction due to acute myocardial infarction after successful reperfusion (48, 49).

Antihypertensive effects of GLP-1 in salt-sensitive Dahl rats have been demonstrated (50), but blood pressure is modestly reduced by GLP-1 in animal models and in human beings.

GLP-1 has no effect on glomerular filtration rate in healthy subjects, but it reduces glomerular hyperfiltration in obese, insulin-resistant subjects (51); it also increases sodium and reduces H⁺ excretion, suggesting a direct effect on the Na⁺/H⁺ transporter in the proximal tubule (52). Although it has no effect on renin, angiotensin II or aldosterone concentrations in healthy people (51), its acute administration induces natriuresis and diuresis in lean and obese insulin-resistant subjects, including people with T2DM (52).

Administration of exendin 4, a DPP-4 resistant GLP-1 analogue, ameliorates hepatic steatosis and liver oxidative stress markers in ob/ob hyperglycaemic mice (53).

The lipid profile, mainly postprandial triglycerides, is favourably changed (even modestly) in humans (4).

**Glucose-dependent insulinotropic polypeptide.**

It is a single 42 aminoacid peptide synthetized by enteroendocrine K-cells of the proximal intestine (54). Although formerly called gastric inhibitory peptide (GIP), since its main effect is insulinotropic, it was renamed glucose-dependent insulinotropic polypeptide.

GIP belongs to the family of secretins, presenting homology with some of its members: secretin, glucagon, GLP-1 and 2, VIP, and GRRH. As most of them, GIP has a synthesis precursor of higher molecular weight, a 153-aminoacid molecule encoded by the gip gene (55).

Food ingestion stimulates the intestinal release of GIP in a magnitude proportional to the amount of food ingested (56); in humans, the stimulatory effect of fat is higher than that of carbohydrates (57). Nevertheless, oral fat alone (without carbohydrates), even when it induces GIP release, is not sufficient to stimulate insulin secre-
tion. This could be explained by the fact that the effects of GIP on insulin secretion do not occur if blood glucose levels are not above fasting levels (4).

Once released, GIP is rapidly degraded by DPP-IV to an inactive truncated derivative (58). Inhibition of DPP-IV activity increases GIP levels, decreases glycemia in people with T2DM (59) and delays the appearance of diabetes in Zucker rats (60).

GIP receptor is a glycoprotein associated to a G protein which activates adenylate cyclase with the subsequent increase of cAMP that can further act through a PKA-dependent and a PKA-independent pathway. In the latter, GEFII-Rim2 acts as a cAMP mediator, as it occurs with GLP-1 (29). It also acts by opening voltage-dependent Ca\(^{2+}\) channels, thus increasing cytosolic Ca\(^{2+}\) and activating phosphatidyl inositol 3-kinase (PI3-K) and MAP kinases (61, 62). GIP exerts its insulinotropic effect through the adenilate cyclase/cAMP/PKA cascade (61, 62).

GIP stimulates insulin secretion, proinsulin gene transcription (63) and Pdx-1, GLUT2 and glucokinase gene expression (64). It also stimulates the differentiation, replication, growth and proliferation of pancreatic β-cells (65), inhibiting their apoptosis (66).

GIP presents functional extrapancreatic receptors in liver, muscle, adipose tissue, intestine and in central (CNS) and sympathetic nervous system (SNS). Therefore, GIP inhibits hepatic glucose production (67), glucose uptake by muscle (68), glucose transport in adipose tissue (69), and fatty acid synthesis (70) in adipocytes. Intestinal infusion of GIP increases GLP-1 and somatostatin secretion (33).

GIP receptors were found in the CNS, at the hippocampus and at hippocampal progenitor cells. In several animal models, GIP mRNA and/or protein are expressed in the olfactory system, hippocampus, amygdala, Purkinje cells, striatum, thalamus, hypothalamus, retina and brainstem (4). GIPR/- knockout animals display reduced neural progenitor cell proliferation and behavioural alterations.

GIP plasma concentrations seem to be elevated in obese subjects: as mentioned before, GIP receptor are located on fat cells, and their number increases during the preadipocyte to adipocyte differentiation process. It inhibits glucagon-stimulated lipolysis and enhances lipoprotein-lipase activity (71). In isolated adipocytes, it potentiates the insulin effect by increasing glucose uptake and fatty acid storage.

The physiological importance of GIP activity was confirmed using mice with KO of its receptor gene. These mice developed glucose intolerance, decreased insulin secretion and were resistant to develop obesity when they were fed a fat-rich diet (21). On the other hand, KO of GIP receptors in ob/ob mice caused weight loss with improved adiposity and glucose tolerance. On account of this evidence, it has been postulated that people with increased GIP response are prone to develop obesity and hyperinsulinism. People with T2DM develop GIP resistance; therefore, insulin secretion decreases in response to oral glucose, which affects primarily second-phase of insulin secretion (31). In view of the therapeutic use of GIP in people with T2DM and considering its short half-life, analogues with activity higher than that of the native molecule have been developed (72, 73).

Interestingly, GIP demonstrated intense effects on bone. GIP receptors can be found on osteoblasts, osteocytes and osteoclasts. GIP administration leads to a reduction in bone resorption in culture systems (74). Under GIP exposure, osteoblast-like cells increase cAMP, intracellular calcium content and Type I collagen expression (75). Transgenic mice over-expressing GIP have increased bone mass and decreased markers of bone resorption (76). GIP receptor knockout animals have a reduced bone mass and reduced bone turnover (77).
CONCLUSIONS.

A better understanding of GLP-1 and GIP biology, physiology and pathophysiology are currently within the focus of many labs across the world, since they are in the base-ment of the development of new therapies for treating type 2 Diabetes and obesity. Many of the main aspects of the incretins physiology are naturally complex, because a myriad of pleitropic actions, involving several biological systems, displaying marked interspecies and inter-individual variability.

The search for new regulatory mechanisms involving the entero-insular axis is also extremely active, and novel promising compounds are currently being studied.

Even when it results impossible to predict the long term success of the pharmacol-ogical intervention on these new targets, further advances in this area are granted.

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