

Physiology of Incretins in Health and Disease

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■ Abstract

The incretin hormones, glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1), are gut peptides which are secreted by endocrine cells in the intestinal mucosa. Their plasma concentrations increase quickly following food ingestion, and carbohydrate, fat, and protein have all been shown to stimulate GLP-1 and GIP secretion. Although neural and hormonal mechanisms have also been proposed to regulate incretin hormone secretion, direct stimulation of the enteroendocrine cells by the presence of nutrients in the intestinal lumen is probably the most important factor in humans. The actions of the incretin hormones are crucial for maintaining normal islet function and glucose homeostasis. Furthermore, it is also now being rec-

ognized that incretin hormones may have other actions in addition to their glucoregulatory effects. Studies have shown that GLP-1 and GIP levels and actions may be perturbed in disease states, but interpretation of the precise relationship between disease and incretins is difficult. The balance of evidence seems to suggest that alterations in secretion and/or action of incretin hormones arise secondarily to the development of insulin resistance, glucose intolerance, and/or increases in body weight rather than being causative factors. However, these impairments may contribute to the deterioration of glycemic control in diabetic patients.

Keywords: DPP-4 · GIP · GLP-1 · glucose-dependent insulintropic polypeptide · glucagon-like peptide-1 · glycemic control · gut hormone · incretin · obesity · type 2 diabetes

Introduction

Incretin hormones are gut peptides secreted in response to nutrient ingestion, which play a key role in the regulation of islet function and blood glucose levels. In humans, the major incretin hormones are glucagon-like peptide (GLP)-1 and glucose-dependent insulintropic polypeptide (GIP), and together they fully account for the incretin effect [1]. The incretin effect is defined as the phenomenon whereby orally ingested glucose elicits a much greater insulin response than that obtained when glucose is infused intravenously to give identical blood glucose levels (the so-called isoglycemic glucose infusion). Depending on the size of the stimulus, the incretin effect can account for up to 70% of glucose-induced insulin secretion

in healthy humans [2]. It is explained by the fact that oral, but not intravenous, glucose stimulates the release of the incretin hormones which then enhance glucose-stimulated insulin secretion.

Interestingly, a similar phenomenon has recently been reported for lipids, with oral ingestion being associated with increased insulin (and incretin hormone) responses compared with matched intravenous lipid infusions [3]. GIP is produced in the K-cells, located predominantly in the more proximal parts of the small intestine, with highest density in the duodenum. In contrast, GLP-1 is produced by the more distally situated L-cells, primarily in the ileum, and is also found in high density in the colon [4]. However, both cell types can be found throughout the whole intestine, and there is also evidence that a population of

cells exists in which both GLP-1 and GIP are co-localized [5].

Both K- and L-cells are “open-type” endocrine cells located in the intestinal mucosa, meaning that they can be influenced by direct contact with nutrients derived from food ingestion. It has been proposed, however, that other mechanisms are also involved in regulating the secretion of GLP-1 and GIP. Once released, both hormones are rapidly degraded by the enzyme dipeptidyl peptidase 4 (DPP-4) [6, 7]. The discovery that DPP-4 has a key role in the degradation of GLP-1 has led to the development of DPP-4 inhibitors as therapeutic agents in the management of type 2 diabetes [8, 9].

Incretin hormone biosynthesis and metabolism

GLP-1 is derived from the post-translational processing of the proglucagon gene product by prohormone convertase 1/3 (PC-1/3) in the intestinal L-cells. This process also results in production of the related peptide GLP-2. In the pancreatic α -cell, the action of PC-2 gives rise to glucagon [10]. However, recent studies suggest that, under some circumstances, small amounts of GLP-1 may also be produced in the α -cell, with isolated rat and human islets being shown to contain GLP-1 following exposure to high glucose concentrations [11, 12]. In the L-cell, proglucagon processing generates a 31-residue peptide, designated GLP-1(7-37). This peptide can be amidated at the C-terminal glycine residue to give rise to a second form, GLP-1(7-36)NH₂. In humans, the amidation process is efficient, so that the majority of the pep-

tide released from the L-cell is in the amidated form [13].

In the case of GIP, PC-1/3 processing of pro-GIP within the K-cells results in the formation of a single bioactive 42-residue peptide [14]. As for GLP-1, there is some evidence to suggest that α -cells may also be able to produce small amounts of GIP, which undergoes processing by PC-2 to form a C-terminally truncated peptide, GIP(1-30) [15]. In the intestinal enteroendocrine cells, both GLP-1 and GIP are stored as intact peptides within secretory granules until they are secreted. Once released, N-terminal degradation by DPP-4 forms the metabolites, GLP-1(9-37)/GLP-1(9-36)NH₂ and GIP(3-42), which account for the majority of the circulating forms of the two incretins [6, 7]. This degradation is a rapid process, giving the intact peptides short half-lives of only 1-2 minutes (GLP-1) [16, 17] and 2-3 minutes (GIP) [7, 18].

Incretin hormone secretion

Direct luminal stimulation of enteroendocrine cells

Incretin hormone release is strongly correlated with food intake [19, 20]. Plasma concentrations rise quickly; increases are already apparent within minutes after nutrient ingestion, and remain above basal levels for several hours [21]. The driving factor for stimulating incretin hormone secretion seems to be the presence of nutrients within the intestinal lumen rather than distension of the stomach, since ingestion of a water load only results in a minimal increase in GLP-1 and GIP concentrations [22]. The release of GLP-1 is related to the rate of gastric emptying and the appearance of nutrients in the intestinal lumen [23, 24]. In contrast, GIP release seems to be dependent on the rate of absorption rather than the mere presence of nutrients [24, 25]. A number of studies have shown that all three macronutrients (carbohydrate, protein, and fat) are capable of stimulating the release of the incretin hormones (reviewed in [22, 26]), although there does seem to be some difference in the response patterns of each of these three nutrients. Carbohydrates are a good stimulus for both GLP-1 and GIP secretion [19, 21]. However, while the GLP-1 response is similar after ingestion of isocaloric fat or protein loads, protein appears to be a more potent stimulus than fat for the early release of GIP [27].

The precise mechanism by which macronutrients cause release of the incretin hormones is not

Abbreviations:

AMP - adenosine monophosphate
ATP - adenosine triphosphate
BMI - body mass index
cAMP - cyclic adenosine monophosphate
CVD - cardiovascular disease
DPP-4 - dipeptidyl peptidase-4
GABA - gamma-aminobutyric acid
GIP - glucose-dependent insulinotropic polypeptide
Glc - glucose
GLP-1 - glucagon-like peptide-1
GPR - G-protein-coupled receptors
K - potassium
Na - sodium
NEFA: non-esterified fatty acid
PC1/3 - prohormone convertase 1/3
SGLT-1 - sodium-glucose cotransporter 1
TGR5 - G-protein-coupled bile acid receptor 5 (also called Gpbar-1)

fully understood, but it is believed that direct interaction between the ingested nutrients and the K- and L-cells may be the principal mechanism involved (Figure 1). *In vitro* studies have demonstrated that changes in membrane potential and mobilization of intracellular calcium following glucose stimulation lead to GLP-1 and GIP release [28, 29]. Several glucose-sensing pathways have been suggested to be involved, including active glucose uptake via the sodium-glucose co-transporter SGLT-1 [29-31], and closure of ATP-sensitive K^+ channels [29, 32]. More recently, a mechanism involving activation of the sweet taste-receptor, gustducin, has also been proposed [33]. However, it is still unclear whether all of the above mentioned pathways are involved in mediating glucose-induced incretin hormone secretion in humans, since neither sulphonylureas nor ingestion of non-caloric non-metabolizable sweeteners have been observed to affect incretin levels in clinical studies [34-36]. It has been thought that glucose was able to stimulate GLP-1 secretion only via interaction with the apical (luminal) membrane of the L-cell, as intravenously infused glucose was not thought to result in elevated GLP-1 levels. However, GLP-1 secretion from the isolated perfused intestine was stimulated by high glucose concentrations in the perfusate, suggesting that glucose in the blood may be capable of stimulating GLP-1 release as well [37]. This suggestion has been supported by the development of more sensitive GLP-1 assays. Using these assays, it has been possible to demonstrate that hyperglycemia itself can lead to small, but significant, increases in GLP-1 levels [38]. However, the mechanism by which circulating glucose stimulates the L-cells remains unknown.

The mechanisms mediating the effects of other macronutrients on GLP-1 and GIP secretion have

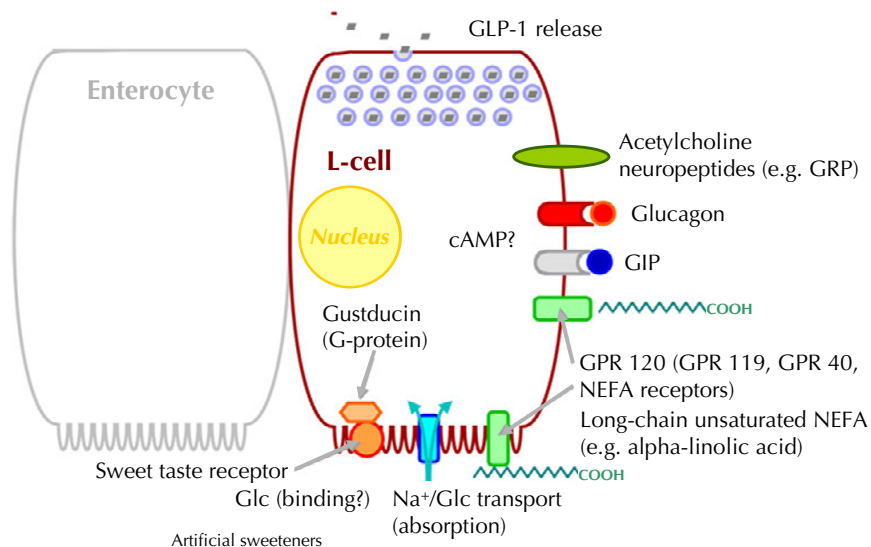


Figure 1. Proposed mechanisms involved in stimulating L-cell secretion. Absorption of nutrients over the luminal (brush border) membrane may be the predominant mechanism in humans, although other signals may include neurotransmitters, hormones and bile acids (see text for details). cAMP: cyclic adenosine monophosphate. GIP: glucose-dependent insulinotropic polypeptide. Glc: glucose. GLP-1: glucagon-like peptide-1. GRP: G-protein-coupled receptor. Na: sodium. NEFA: non-esterified fatty acid. Reproduced with kind permission from [122] (Springer Science+Business Media; Diabetologia, 2011, 54:10-18, Nauck et al., Fig. 1).

not been well characterized. Amino acids such as alanine, glutamine, and asparagine can stimulate GLP-1 secretion via mechanisms which may involve activation of ligand-gated ion channels or sodium-coupled uptake. Both pathways lead to membrane depolarization, calcium influx, and finally GLP-1 release [39-41]. Enteroendocrine cells express a number of different G-protein-coupled receptors (GPR) [31, 42], some of which have been proposed to mediate the stimulatory effects of dietary fats on incretin hormones. Naturally occurring lipid amides, such as oleoylethanolamide, and 2-monoacylglycerols, such as 2-oleoyl glycerol, have been shown to act as endogenous ligands of GPR119 [43, 44]. Activation of GPR119 is associated with increased cyclic AMP levels and GLP-1 release in mice [45] and humans [44]. Other GPR have been identified which are responsive to long-chain fatty acids, (e.g. GPR40, GPR120) and which have been suggested to mediate free fatty acid stimulation of GLP-1 [46, 47].

Lipids may also indirectly stimulate GLP-1 secretion via bile acids. Thus, activation of the bile acid receptor TGR5 promotes GLP-1 secretion

from an enteroendocrine cell line *in vitro* [48], and GLP-1 release from the perfused rat colon increases following perfusion with bile acids [49]. Furthermore, preliminary data have shown that intra-rectal infusion of bile acids is associated with increased GLP-1 levels in patients with type 2 diabetes [50]. However, the precise physiological importance of this pathway in humans is unclear, with another preliminary report indicating that postprandial GLP-1 responses are not impaired in cholecystectomized patients [51].

While both K- and L-cells undoubtedly respond to direct stimulation, it has been argued that other mechanisms must be involved to explain the early rise in incretin levels, particularly for GLP-1, where plasma concentrations increase before nutrients reach the more distally located L-cells.

Neural regulation

A number of studies suggest that incretin hormone secretion can be influenced by autonomic nerves, with *in vitro* and animal studies indicating

that vagal cholinergic muscarinic pathways are involved [26]. In rodents, a transient increase in GLP-1 levels was observed in anticipation of food being presented [52], suggesting a cephalic response. However, in humans, the balance of evidence suggests that vagal cholinergic innervation does not play a major role in regulating incretin hormone secretion. Sham-feeding does not alter GLP-1 concentrations [53], while basal GLP-1 and GIP concentrations remain unchanged in the pre-absorptive (cephalic) period [54], and are unaffected by the non-specific muscarinic blocker atropine. Furthermore, there are no major changes in prandial responses following small intestinal resection [55]. A role for the sympathetic nervous system and for non-adrenergic non-cholinergic neurons has been suggested from animal studies. However, it

Table 1. Actions of GLP-1 and GIP in the different organs

Organ	GLP-1	GIP
<i>Pancreas</i>		
	Stimulates glucose-dependent insulin release	Stimulates glucose-dependent insulin release
	Upregulates insulin gene expression and all steps in insulin biosynthesis	Upregulates insulin gene expression and all steps in insulin biosynthesis
	Upregulates expression of other genes (e.g. glucokinase, GLUT-2) essential for β -cell function	Upregulates expression of other genes essential for β -cell function
	Enhances β -cell proliferation and survival (animal models and isolated human islets)	Enhances β -cell proliferation and survival (animal models and isolated human islets)
	Inhibits glucagon secretion in a glucose-dependent manner	Stimulates glucagon secretion
<i>Gastrointestinal tract</i>		
	Delays gastric emptying	Minimal effect on gastric emptying
<i>Central and peripheral nervous system</i>		
	Reduces appetite and food intake, leading to weight loss	No significant effect on appetite or body weight
	Neuroprotection (rodents)	
	Improved learning and memory (rodents)	
<i>Cardiovascular system</i>		
	Cardioprotection	
	Improves myocardial function	
	Improves endothelial dysfunction	
	Anti-atherosclerotic effects (rodents)	Anti-atherosclerotic effects (rodents)
<i>Bone</i>		
	May have indirect anabolic effects (rodents)	Anti-osteoporotic effects (rodents)
<i>Adipose tissue</i>		
		Lipogenic and lipolytic effects (rodents)

remains unclear whether these pathways play a significant role in the regulation of incretin hormone secretion in humans [26].

Hormonal regulation

An endocrine loop linking the duodenum to the ileum has also been proposed as a mechanism to explain the paradoxically early rise in GLP-1 levels. GIP, in particular, was shown to be capable of increasing GLP-1 release in a several animal studies, but a role for hormones in stimulating the secretion of GLP-1 in humans has not been established [26]. However, there is some evidence to support the notion that local paracrine mechanisms may act to restrain incretin hormone secretion. It has been observed that endogenous GLP-1 levels are suppressed following infusion of exogenous GLP-1 in humans [56]. Furthermore, DPP-4 inhibition, which leads to increased levels of endogenous intact (active) GLP-1 and GIP, is associated with lower total incretin levels, in both animals [57] and humans [34], suggesting that their secretion has been suppressed. These observations support the suggestion that there may be a local feedback loop, whereby the increased levels of intact incretin hormones restrain further secretion [57]. It has been proposed that this is mediated by somatostatin from neighboring δ -cells exerting a paracrine inhibitory effect [58].

The most important stimulus for incretin hormone release in humans is probably the direct stimulation of K- and L-cells, with the explanation for the rapid early increase in GLP-1 level following food ingestion likely to be due to the stimulation of the proximally located L-cells. Notably, surgical removal of the distal intestine has not been observed to affect the GLP-1 response to glucose [55]. It has been shown that nutrients first reach the proximal duodenum within 6-8 minutes following ingestion of a liquid glucose load, which correlates well with the initial increase in GLP-1 levels [24]. Calculation of the amount of GLP-1 present in the upper intestine indicates that this is more than sufficient to account for the GLP-1 response to oral glucose [59]. The extent of the GLP-1 response is related to both meal composition and meal size [60]. Moreover, there is also a diurnal variation, with responses being greater when an identical meal is served in the morning compared to the afternoon [61].

Incretin hormone action

The role conventionally assigned to incretin hormones is that of enhancing glucose-stimulated

insulin secretion. However, both GLP-1 and GIP have multiple actions which influence blood glucose levels beyond simply stimulating insulin secretion. Moreover, it is becoming increasingly clear that they exert a number of other effects in addition to those involved in the regulation of glucose homeostasis (Table 1).

The actions of GLP-1 are mediated by specific GPR found in its target organs such as the pancreas, brain, heart, kidney, and lung, but the presence of the GLP-1 receptor in other tissues such as liver, muscle, and fat cells remains controversial [62]. Many of the peptide's effects in these other tissues are likely to be indirect, at least in humans. GLP-1 has potent effects on pancreatic β -cells; it enhances the meal-stimulated release of insulin (the "incretin effect") in a glucose-dependent manner, stimulates all stages of insulin biosynthesis, and improves overall β -cell function [62]. Moreover, animal and *in vitro* studies have demonstrated that it has a β -cell protective effect, promoting differentiation and proliferation, while reducing apoptosis, which results in increased β -cell mass [62]. However, effects on β -cell mass have not yet been demonstrated in humans. GLP-1 also potently suppresses α -cell secretion of glucagon, again in a glucose-dependent manner. However, reports of GLP-1 receptor expression on α -cells have been inconsistent [63, 64], and the glucagonostatic effect of GLP-1 is believed to be an indirect effect, mediated by insulin, zinc, or GABA released from the β -cells [65, 66], and/or somatostatin released from the neighboring δ -cells [67]. Together, the insulinotropic and glucagonostatic effects of GLP-1 have been shown to exert a powerful effect on blood glucose levels. Studies using the GLP-1 receptor antagonist exendin 9-39 have revealed that endogenous GLP-1 is important for the regulation of normal glucose homeostasis. Thus, when infused into healthy humans, exendin 9-39 was associated with increased postprandial glucose excursions [68]. Since this finding, several clinical studies have demonstrated that endogenous GLP-1 plays a major role in regulating pancreatic islet responses to glucose [69, 70]. When GLP-1 action is blocked glucose-induced insulin secretion is lower and the incretin effect is reduced by around 50% [69]. Both the stimulation of insulin and the suppression of glucagon secretion are important mediators, and have been demonstrated to contribute almost equally to the glucose-lowering effect of GLP-1 [71].

GLP-1 also reduces gastric emptying, which has been proposed to be the principal mechanism by which GLP-1 reduces postprandial glucose ex-

cursions [72]. Nevertheless, it remains unclear how much this mechanism contributes to the glucose-lowering effect of endogenous GLP-1, since GLP-1 receptor antagonism using exendin 9-39 affected gastric emptying in some [73], but not all [70, 74], studies.

Numerous studies have indicated that GLP affects appetite and food intake in humans [75], with both peripheral and central mechanisms being proposed as mediators [62]. Exogenous GLP-1 and GLP-1 receptor agonists used clinically have consistently been associated with body weight loss, whereas enhancement of endogenous GLP-1 levels using DPP-4 inhibitors is generally body weight neutral [75, 76]. This may suggest that higher GLP-1 concentrations are necessary to elicit the body weight-lowering effect than are required for the antihyperglycemic effects. However, the extent to which GLP-1 plays a physiological role in regulating appetite/satiety and body weight is unclear. In rodent studies, investigating the role of GLP-1 on food intake, GLP-1 receptor antagonism using exendin 9-39 has given conflicting results [77-79].

GLP-1 receptors are also found throughout the cardiovascular system, and data are accumulating from both animal and clinical studies which suggest that both exogenous and endogenous GLP-1 may be cardioprotective and have beneficial cardiovascular actions. These actions are reviewed elsewhere in this issue [80, 81]. There is also pre-clinical evidence that GLP-1 may exert neuroprotective effects. GLP-1 receptors have been demonstrated on both peripheral and central neurons, and favorable effects of GLP-1 have been observed in rodent models of Parkinsonism and Alzheimer's disease (reviewed in [82]). Moreover, recent data from animal studies suggest that GLP-1 may have the potential to ameliorate peripheral neuropathy. GLP-1 receptor activation was reported to be associated with both histological and functional improvements in a rat model of pyridoxine-induced peripheral sensory neuropathy [83]. Diabetes-induced peripheral nerve degeneration was reduced in studies using a GLP-1 receptor agonist or a DPP-4 inhibitor to enhance endogenous GLP-1 levels [84, 85], while hyperglycemia-induced reductions of motor nerve conduction velocity were attenuated by GLP-1 receptor agonism in diabetic mice [86]. However, as yet, no studies have addressed potential neuroprotective properties of GLP-1 in humans.

Like the GLP-1 receptor, the GIP receptor is also a GPR, found in numerous tissues, including

pancreas, bone, brain, and adipocytes [62]. GIP shares many of the GLP-1 effects on the β -cell, where it increases insulin biosynthesis and secretion and exerts a β -cell protective effect [62]. However, its effect on the α -cell contrasts with that of GLP-1; GIP stimulates the release of glucagon from the perfused rat pancreas [67], and has glucagonotropic actions in humans [87]. GIP has been implicated in lipid metabolism and adiposity from studies in rodents [62]. Knock-out of the GIP receptor confers resistance to diet-induced obesity in mice [88], although there is no clear evidence at present to suggest an association between GIP and obesity in humans. In bone, GIP receptors are located on both osteoclasts and osteoblasts, and studies in mice and rats have indicated that GIP signaling is involved in the regulation of bone turnover. Bone formation is enhanced while bone resorption is suppressed, which leads to increases in bone mass and bone mineral density [89]. However, although the GIP receptor has been demonstrated in some human bone cell-lines [90], acute administration of GIP has not been associated with changes in markers of bone resorption in humans [91].

Incretin hormones in disease states

Interpretation of the precise relationship between disease and incretin hormone secretion and the incretin effect is complicated. However, there is evidence to suggest that impairments in secretion and/or action of incretin hormones arise secondarily to the development of insulin resistance, glucose intolerance, and/or increases in body weight rather than being causative factors. In separate studies, insulin sensitivity, glucose tolerance, and body mass index (BMI) have all been identified as independent factors associated with reductions in GLP-1 secretion and an impaired incretin effect [92-94].

Obesity

The incretin effect has been reported to be reduced in obese subjects with normal glucose tolerance [94, 95], but it is unclear whether this is due to reduced secretion of incretin hormones and/or impairments in their action. After mixed meal and glucose ingestion, the secretion of GLP-1, but not of GIP, is reduced in obese subjects compared to lean subjects [27]. There is also evidence for a link between body weight and GLP-1 from a number of studies, all showing an inverse relationship between body weight and GLP-1 levels [27, 93, 94,

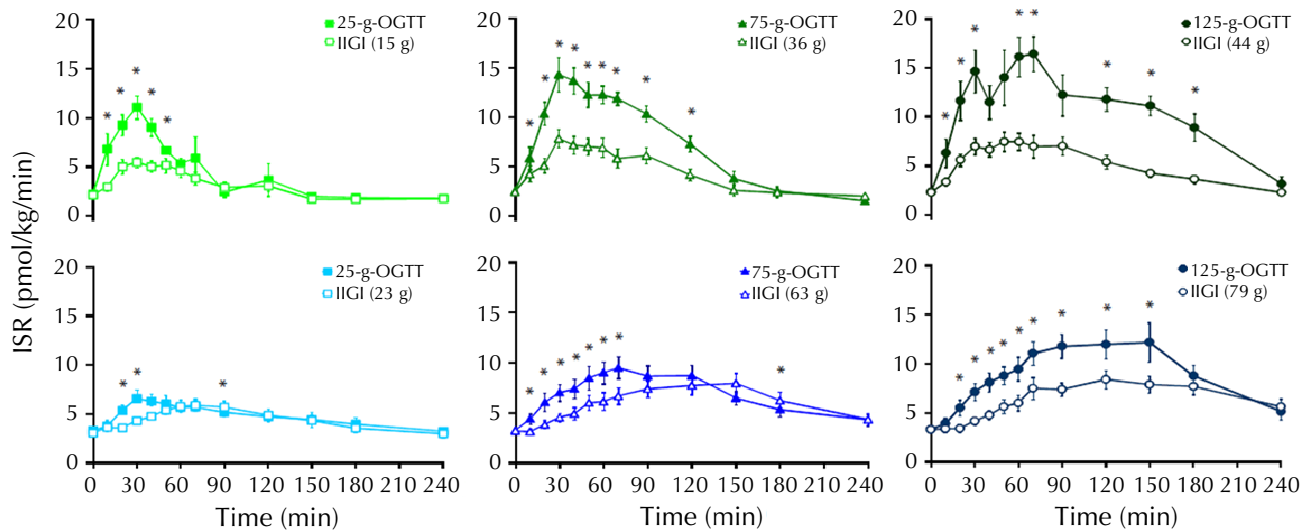


Figure 2. Impaired regulation of the incretin effect in patients with type 2 diabetes. Insulin secretion rates (ISR) in healthy control subjects (upper panel) and patients with type 2 diabetes (lower panel) following low (25 g), medium (75 g), and high (125 g) oral glucose loads (closed symbols) and corresponding isoglycemic intravenous glucose infusions (open symbols). Figures in brackets indicate the amount of glucose infused to mimic the glycemic excursion following the oral glucose. * $p < 0.05$. IIGI: isoglycemic intravenous glucose infusion. ISR: insulin secretion rate. OGTT: oral glucose tolerance test. Reproduced with kind permission from [110] (The Endocrine Society, *J Clin Endocrinol Metab*, 2011, 96:373-345, Bagger et al., Fig. 2c).

96]. Meal-stimulated GLP-1 levels are impaired in obese subjects with normal glucose tolerance compared with lean subjects, with some partial recovery in levels following weight loss [97]. However, there is little consensus regarding the relationship between obesity and GIP secretion. Most studies have found no impact of changes in body weight on GIP levels [27, 94, 97], although in other studies, both modestly reduced [93] and increased [98] GIP levels have been reported in obese subjects.

At present, only a few studies have addressed the subject of whether incretin hormone action is altered by obesity. One study has shown that the insulinotropic ability of GIP is similar in obese and lean subjects [99]. In contrast, an animal study demonstrated an augmented insulinotropic action of GLP-1 in high-fat fed obese and insulin-resistant mice compared with normal mice [100].

Subjects at risk of developing type 2 diabetes

Normal glucose-tolerant subjects at increased risk of subsequently developing diabetes have been studied to find out whether impaired incretin secretion and/or action is apparent before overt diabetes appears. Incretin hormone concentrations appear normal in non-diabetic offspring of type 2 diabetes patients [101] and in first-degree rela-

tives of diabetic subjects [102], whereas, in identical twins discordant for diabetes, reduced GLP-1 levels were observed in the affected twin only [103]. In women with gestational diabetes, impaired GLP-1 responses have been reported [104], but these return to normal following birth and re-establishment of normal glucose tolerance [104, 105].

Data are conflicting regarding alterations in incretin hormones in subjects with impaired glucose tolerance. Normal GLP-1 responses were reported in some subjects with impaired glucose tolerance [96, 106], whereas an impairment was identified in others. However, further analysis concluded that the reductions could largely be explained by differences in BMI and/or insulin sensitivity [93, 107]. In terms of incretin action in impaired glucose-tolerant subjects, the insulinotropic effect of GLP-1 was found to be reduced [108], and an impaired incretin effect has been reported [107]. In another study, GIP action was normal in subjects with previous gestational diabetes [105].

Type 2 diabetes

In patients with type 2 diabetes, the incretin effect is clearly reduced [109, 110], which results in an inappropriately low insulin response to the

ingestion of nutrients (Figure 2). Several early studies indicated that the reduced incretin effect could, at least in part, be related to impaired secretion of GLP-1 (whereas secretion of GIP is generally found to be unaltered). Impaired meal-stimulated GLP-1 levels have been reported in some studies of patients with type 2 diabetes [93, 94, 111]. However, in other studies, no such impairment was observed [96, 112]. Moreover, it is now being recognized that some anti-diabetic agents themselves are able to influence incretin hormone levels. This finding further complicates the interpretation of data from different studies in which incretin levels were assessed. In this regard, the different treatment regimens are important. In some studies, patients were still on drug therapy whereas in others, therapy had been discontinued and patients were studied after washout periods of differing duration.

It is now well established that DPP-4 inhibitors increase intact incretin hormone concentrations [113]. However, metformin is also associated with increased GLP-1 responses to food ingestion in animals [114] and humans [115], at least in acute studies, although the mechanism has not been fully elucidated. Metformin is not a competitive inhibitor of DPP-4, but results from preclinical studies have suggested that it may upregulate proglucagon expression in the intestinal L-cells [115]. Furthermore, preliminary data have indicated that metformin may also influence GLP-1 levels indirectly, mediated via increased bile acid levels. In mice, metformin was reported to inhibit the apical sodium-dependent bile acid transporter (ASBT), resulting in increased bile acid concentrations in the intestine and increased plasma GLP-1 levels [116]. In accordance with this finding, it has recently been reported that bile acid sequestrants

such as colesvelam have been associated with increased plasma GLP-1 concentrations [117, 118]. Other studies have demonstrated that α -glucosidase inhibitors such as acarbose and miglitol also stimulate GLP-1 secretion in humans [119, 120]. Again, the mechanism is unclear, but may involve increased exposure of the L-cells to nutrients. On the other hand, it has also been reported that in mice, α -glucosidase inhibition is associated with elevated proglucagon gene expression and GLP-1 content in the intestine [121]. Thus, while it appears that some patients with type 2 diabetes are characterized by a reduction in meal-related GLP-1 responses, this may not be a universal trait common to all patients. In particular those with well controlled glycemia may have relatively normal GLP-1 levels (Figure 3) [122].

There does seem to be general agreement that the actions of the incretin hormones are impaired in type 2 diabetes. Although GLP-1 retains its insulinotropic effect in patients with type 2 diabetes, its potency is reduced [123, 124]. In contrast, the actions of GIP are severely impaired [124-126], with an almost complete loss of amplification of the second-phase insulin response. However, it seems unlikely that the impaired incretin effect itself is the cause of type 2 diabetes. Rather, it

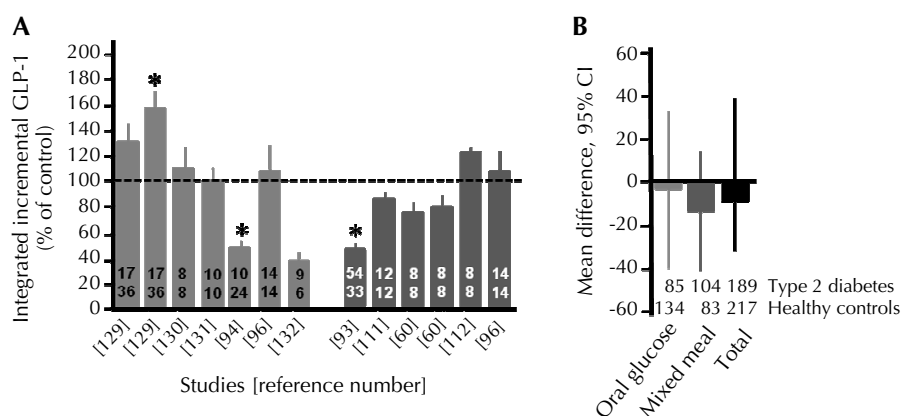


Figure 3. GLP-1 response to glucose. **A:** Integrated “total” GLP-1 response to oral glucose (light grey bars) or mixed meal (dark grey bars) in type 2 diabetic patients, expressed as percentage of the response in matched non-diabetic control subjects. Figures in the bars refer to the number of type 2 diabetic patients (upper row) or control subjects (lower row) studied. Figures below the bars refer to the original studies. **B:** Meta-analysis of published studies reporting GLP-1 responses in healthy individuals and subjects with type 2 diabetes after a 75g oral glucose tolerance test and/or a standardized mixed meal. GLP-1: glucagon-like peptide-1. CI: confidence interval. Modified and reproduced with kind permission from [122] (Springer Science+Business Media, Diabetologia, 2011, 54:10-18, Nauck et al., Fig. 2b).

would appear to develop subsequently to insulin resistance, bodyweight, and/or glucose intolerance, which may contribute to the deterioration of glycemic control in diabetic patients. In accordance with this consideration, similar impairments in incretin actions are seen in diabetic patients irrespective of etiology or phenotype [127]. Moreover, in healthy subjects, an impaired incretin effect has been reported, following short-term (12 days) glucocorticoid treatment to induce reduced glucose tolerance and insulin resistance [128]. Conversely, strict glycemic control to bring about near-normalization of blood glucose levels in patients with type 2 diabetes leads to improvements (although not normalization) in the ability of GLP-1 and GIP to enhance insulin secretion [124].

Conclusions

Incretin hormone secretion is regulated by a variety of different signaling pathways, which allow a coordinated response to a physiological stimulus. Many factors (nutrients, nerves, hormones, and even drugs) can affect K- and L-cell responses via direct and indirect mechanisms. However in humans, direct stimulation by nutrients in contact with the apical membrane of the endocrine cells appears to be the principal factor determining incretin hormone secretion.

Alterations in GLP-1 and GIP responses are reported to be related to changes in body weight, insulin resistance, and glucose tolerance, but the precise ways in which these factors impact on the enteroendocrine cells remains unclear. Nevertheless, changes in incretin hormone levels have been reported in obese glucose-tolerant subjects, with impairments in incretin action becoming evident as glycemic control deteriorates. These impairments probably contribute to the ongoing reduction in β -cell function and developing hyperglycemia observed when overt diabetes becomes established.

Emerging data are suggesting that incretin hormones also have other actions in addition to their accepted roles in the regulation of normal islet function and glucose homeostasis. These include potentially beneficial cardiovascular and neuroprotective actions. However, while the glucoregulatory actions of the incretins have been convincingly demonstrated to be impaired in subjects with diabetes, it is currently unknown whether this also applies to any of their non-glucose effects. Given the increasing use of incretin-based therapies as part of the treatment paradigm for type 2 diabetes, further investigation of the pleiotropic effects of the incretin hormones in disease states is clearly warranted.

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