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MINI REVIEW

Searching for the physiological role of glucose-dependent insulinotropic polypeptide

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ABSTRACT

Glucose-dependent insulinotropic polypeptide (GIP) was established as a gut hormone more than 40 years ago, and there is good experimental support for its role as an incretin hormone although deletion of the GIP receptor or the GIP cells or GIP receptor mutations have only minor effects on glucose metabolism. Unlike the related hormone, GLP-1, GIP stimulates the secretion of glucagon, which in healthy individuals may help to stabilize glucose levels, but in people with type 2 diabetes may contribute to glucose intolerance. A role in lipid metabolism is supported by numerous indirect observations and by resistance to diet-induced obesity after deletion of the GIP receptor. However, a clear effect on lipid clearance could not be identified in humans, raising doubt about its importance. The GIP receptor is widely expressed in the body and also appears to be expressed on bone cells, and experimental studies in rodent point to effects on bone metabolism. Recent studies revealed pronounced inhibitory effects of GIP on bone resorption markers in humans and suggest that GIP may be (one of the) gastrointestinal regulators of bone turn-over. In support of this, a loss-of-function GIP receptor mutation in humans is associated with a marked increase in fracture risk. The lack of a reliable GIP receptor antagonist contributes to the uncertainty regarding the physiological role of GIP.

INTRODUCTION

Glucose-dependent insulinotropic polypeptide (GIP) was discovered in 1973 as a polypeptide inhibitor of gastric acid secretion, based on studies in dogs with Heidenhahn pouches[1]. These are pouches excised from the major curvature of the stomach and are therefore inherently denervated. Administration to the dogs of extractable GIP inhibited acid secretion from the pouches. Studies from the following years suggested that the inhibitory action was only seen after denervation, and further studies suggested that the ability of GIP to stimulate the release of somatostatin from the stomach explained the lack of activity in the innervated stomach, because vagal activity normally would inhibit somatostatin and promote acid secretion[2]. At any rate, in human studies, an effect of physiological amounts GIP on gastric acid secretion could not be detected under controlled conditions[3]. In the meantime, GIP had been established as an effective promotor of insulin secretion, also in humans[4]. Focus therefore shifted towards this aspect of GIP function and, as a consequence, the peptide was renamed glucose dependent insulilnotropic polypeptide (whereby it would retain its acronym); and it was clearly demonstrated in careful clamp studies to effectively potentiate glucose-induced insulin secretion. However, early preliminary studies with porcine GIP indicated that GIP did not affect insulin secretion in people with type 2 diabetes[5], reducing the enthusiasm for this peptide.

THE INCRETIN HORMONES

Originally, there were many misunderstandings with respect to the role of GIP in glucose metabolism, one of the most stubborn being that glucose levels had to be considerably elevated (to 8 mmol/L and above)[6] for the peptide to be effective. This turned out not to be true. In careful studies[7] in which physiological plasma GIP meal responses were mimicked by infusion, a significant effect on insulin was observed in healthy subjects even at their fasting glucose levels (when these were maintained by clamping) and a progressive insulin response was observed throughout the relevant spectrum of postprandial increases (6–7 mmol/L); in particular, it was noted that at these physiologi-

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cal plasma glucose concentrations the insulin responses were similar in magnitude to those elicited by GLP-1 (separately infused also to reach physiological postprandial levels). It was therefore concluded that in healthy individuals, GIP is an important incretin hormone, contributing equally with GLP-1 to the incretin effect. A prominent difference between the two was noted, however: whereas GLP-1 significantly inhibited glucagon secretion at all glucose levels (beyond the inhibition caused by glucose alone), GIP did not affect glucagon secretion (actually a small, significant increase could be discerned at the fasting plasma glucose concentration).

EFFECTS OF THE INCRETIN HORMONES IN TYPE 2 DIABETES MELLITUS

In people with dysregulated type 2 diabetes, the differences are much more dramatic. During intravenous infusions of GIP and GLP-1, again to reach *physiological* concentrations during the conditions of a hyperglycaemic clamp (necessitated by the high fasting glucose levels of the diabetes patients), neither of the two hormones affected insulin secretion[8]. Glucagon was inhibited similarly by the clamp whether the incretin hormones were present or not. With *pharmacological* infusions of GLP-1 and GIP, important differences emerged. Whereas GIP in huge doses (up to 16 pmol/kg min) did not affect insulin secretion (apart from a short-lived early insulin response, which did not result in changes in glucose turnover), GLP-1 cause an increase, similar to that observed in nondiabetic controls with glucose alone[9]. Glucose turnover (evaluated from the infusion rate required to maintain the clamp) was increased correspondingly. With these doses, GLP-1 inhibited glucagon secretion reaching levels similar to those observed in non-diabetic control subjects, whereas GIP significantly stimulated glucagon secretion. Thus, while GLP-1 had powerful antidiabetic actions in these patients, GIP actually seemed to be diabetogenic. In further studies of the actions of GIP on glucose regulation and pancreatic hormone secretion, Christensen *et al.*[10] reached the conclusion that GIP plays a role to fine-tune glucose levels: in healthy individuals, at high glucose levels, it helps to promote insulin secretion, whereas at low glucose levels it enhances glucagon secretion, but has no effects on insulin secretion. Both actions will tend to stabilize plasma glucose levels within a limited interval.

DOES GIP COEXIST WITH GLP-1 IN GUT ENDOCRINE CELLS?

In spite of data recently accumulated indicating that the traditional classification of gut endocrine cells is no longer tenable[11], and that a spectrum of combinations of the various hormones may be found in most gut endocrine cells[12] (e.g., cells producing both GIP and GLP-1), the majority of GIP producing cells are still found in the proximal small intestine and the duodenum[13], while GLP-1 producing cells are much more disperse, with many cells in both the proximal and distal small intestine. This might suggest that GIP is the 'first-in-line' incretin, but there is no evidence that GLP-1 responses are delayed compared to GIP responses. Studies in isolated perfused proximal small intestine also suggest that GLP-1 and GIP responses to nutrients are elicited simultaneously[13]. GIP and GLP-1 however, were only co-localized in few of the cells. Interestingly, the GLP-1 responses from the proximal half of the small intestine were similar to those from the distal small intestine, whereas PYY (traditionally thought to co-exist with GLP-1 in all L-cells) was only secreted from the distal half, and GIP mainly from the upper half[13]. Further, it could be concluded that he GLP-1 secreting proximal cells must be different from the distal cells, because only the distal secrete PYY (and showed a high degree of co-localization). In further support for the existence of two separate cell types (i.e., K- and L-cells), we recently demonstrated in rodents that only GLP-1 secreting cells, and not the GIP cells, express the bombesin-2 receptor and respond to neuromedin C or bombesin[14].

GIP AND GLUCAGON SECRETION IN TYPE 2 DIABETES

It is well established that glucagon secretion increases paradoxically in patients with type 2 diabetes after oral glucose, whereas after intravenous glucose a normal suppression of secretion is observed[15]. We recently investigated whether secretion of gut hormones might be responsible for this, by infusing physiological amounts of GIP, GLP-1 and GLP-2 or a combination of all (because all of these had previously been found to influence glucagon secretion in humans *in vivo*) to patients with type 2 diabetes mellitus, who also received an intravenous glucose infusion adjusted to copy precisely the glucose response to an oral glucose load[16]. GIP caused a pronounced early glucagon response, whereas GLP-1 inhibited glucagon secretion more than glucose alone, while GLP-2 was relatively inert. The glucagon profile after everything in combination closely resembled the profile observed after oral glucose alone, suggesting that hormones secreted from the gut with an action on glucagon secretion might explain the paradoxical post-OGTT glucagon secretion. On the other hand, we also recently observed a similar hypersecretion of glucagon (verified by mass spectrometry) after an oral glucose load in patients without a pancreas (i. e. after total pancreatectomy), suggesting that the source of the paradoxical response could be the gut itself[17]. A hint of a paradoxical glucagon response to large loads of oral glucose may also be observed in healthy subjects[18] and in patients, who have undergone Roux-en-Y gastric bypass[19]. Further studies are required to resolve this issue.

GIP AND LIPID METABOLISM

It is often assumed that GIP plays a significant role in lipid metabolism[20] by stimulating lipid uptake in adipose tissue, although there is also evidence that GIP may have lipolytic activities[21]. Conceivably insulin could function as a metabolic switch to shift lipogenesis from lipolysis. Support for an essential role for GIP in peripheral lipid uptake came from studies of GIP receptor knockout mice, which, unlike control animals, were resistant to diet-induced obesity[22]. Surprisingly, in other

studies, the body weight difference between GIP knockout and controls appeared to be due to differences in lean body mass rather than fat mass[23]. In recent studies in our laboratory, K-cells were acutely destroyed by diphtheria toxin administration in mice transgenic for the human diphtheria-toxin receptor under the control of the GIP promoter[24]. The purpose was to study the effects of acute lack of GIP on lipid absorption profiles and lipid uptake in all relevant tissues using labelled oleic acid. However, in no case was there any difference between control and K-cell depleted animals in spite of extensive and documented deletion of almost all GIP cells and marked reduction of plasma GIP concentrations (unpublished data).

We previously studied healthy volunteers who received a fixed breakfast meal with and without a superimposed infusion of GIP in amounts sufficient to elevate GIP levels to similar levels as the meal itself[25]. After 4 h an *ad libitum* meal was also served. In spite of the significantly elevated GIP levels throughout the study, there was no difference compared to controls for any of the following parameters: plasma concentrations of glucose, insulin, C-peptide, glucagon, GLP-1, GLP-2, triglycerides, FFAs; energy intake at the *ad libitum* meal; gastric emptying (paracetamol was added to the fixed meal); energy expenditure; and respiratory quotient (RQ) after the fixed meal. In separate studies, it was ascertained that the GIP infusate contained highly bioactive GIP. In view of these surprisingly negative findings, we hypothesized that endogenous GIP, which rose to about 100 pmol/L (an appropriate response in relation to the meal served) had already exerted all the relevant effects of GIP. We, therefore, carried out a study in which endogenous GIP secretion could be avoided, namely by administering nutrients (glucose and a stabilized triglyceride emulsion (Intralipid)) intravenously rather than orally[25]. In these experiments, there was as expected a marked stimulation of insulin secretion when GIP was administered intravenously together with glucose. There were also effects on FFA levels, which followed insulin secretion; but triglyceride clearance (as estimated from the plasma trigyceride profile) was completely unaffected. Thus, two observations supported a lack of effect of GIP on peripheral lipid uptake (clearance): (1) the absence of an effect on triglyceride clearance in the meal experiments (even in the late period, when endogenous GIP had reached basal levels, but GIP was still elevated because of the infusion); and (2) the similar absence of effects of elevated GIP on the levels of exogenous triglycerides infused to levels mimicking meal intake.

In other experiments, lipid uptake in the abdominal subcutaneous adipose tissue was studied directly in humans using the Fick principle (arteriovenous concentrations differences multiplied by adipose tissue blood flow)[26]. In this study a combined infusion of lipid, glucose and GIP (elevating also insulin concentrations) was associated with an increased lipid uptake in the adipose tissue, but the uptake was entirely governed by changes in adipose tissue blood flow rather than increased fractional uptake. Also obese individuals and people with type 2 diabetes were studied, but in these the effect was not demonstrable[27]. The effect in the healthy volunteers was quite large, and it could be calculated that if the uptake in the abdominal subcutaneous adipose tissue compartment, drained by the specific catheterized vein, was extrapolated to the entire subcutaneous fat mass, this uptake would have resulted in a major whole body clearance, which easily would have been observable in the meal and infusion studies discussed above. The findings nevertheless suggest that GIP (in combination with glucose and insulin) may affect adipose tissue blood flow in healthy individuals, and thereby possibly lipid uptake, a finding that deserves further study. We recently examined 1405 individuals at low to high risk of developing type 2 diabetes and found unexpectedly that high fasting GIP levels, independent of insulin, were associated with lower LDL levels, indicating that high *fasting* GIP levels may promote lipid clearance from blood[28]. Thus, regarding the GIP effects on fat metabolism, it seems that we still have a lot to learn.

GIP AND BONE METABOLISM

As discussed above, GIP receptors are present in many tissues and have also been reported to be present in bone cells, including osteoblasts, osteocytes and osteoclasts[29], suggestive of a direct effect of GIP on these cells. *In vitro*, GIP increases cAMP and intracellular Ca^{2+} levels in osteoblasts, leading to increased expression of collagen type I and alkaline phosphatase, indices of bone formation[29]. Furthermore, GIP stimulation of osteoclasts has been reported to reduce PTH-induced bone resorption[30]. This suggests that an 'entero-osseous-axis' may exist, where nutrient-related hormones such as GIP modulate bone turnover to coordinate optimal utilization of nutrients by bone[31]. GLP-2 appears to have similar functions. In accordance with this hypothesis, $GIPR^{-/-}$ mice were reported to have lower bone mineral density (BMD) and bone mineral content (BMC) as well as weaker and less stiff bones[32], but other studies could not confirm a reduced bone growth in the $GIPR^{-/-}$ mice[33], but agreed on the anabolic effects of GIP on bones. The main site of GIP action may be the osteoblasts, on which GIP showed anti-apoptotic effects. However, GIP may also indirectly stimulate bone formation via its action on the pancreatic beta cells[31]. In humans, we previously reported uncertain effects of single subcutaneous injections of GIP on circulating markers of bone resorption (CTX) and formation (osteocalcin)[34], but in a more recent study, we employed intravenous infusions of physiological amounts of GIP alone or with an infusion of glucose mimicking postprandial levels, and found a marked (50%) reduction of CTX by the combination[35]. This reduction is similar to what is observed after ingestion of large meals[34]. Glucose alone had a small effect whereas GIP alone caused about a 30% reduction in CTX levels. Obviously, this raises the question of the relative importance of glucose alone, glucose-stimulated insulin secretion or insulin secretion following the combined GIP and glucose stimulation. The literature about this offers little help and further studies designed to distinguish between these mechanisms are clearly warranted. The results, however, strongly sup-

port the concept of an 'entero-osseous axis', where hormones from the GI tract released by nutrient ingestion, perhaps together with glucose and insulin are responsible for the decrease in bone resorption normally observed during the day; a corresponding increase in bone resorption during the night time, where gut hormone secretion is minimal, restores the homeostatic balance in bone remodelling. As mentioned GLP-2, but not GLP-1, may have a similar function[36], but judged from the CTX reductions, GIP seems more efficacious[37]. The importance of the GI tract for these mechanisms is supported by the absence of a clear effect on the bone markers during the day and night in fasting individuals[34]. Support for a specific role of GIP was provided recently in a study of human carriers of the functional missense GIPR variant Glu354Gln (rs1800437)[38]. In a large meta-analysis, this variant causes slightly but significantly higher glucose levels and slightly lower insulin levels 2 h after an oral glucose tolerance test, but the changes are very small and the risk of type 2 diabetes is only increased by 7%[39]. In our study of a large cohort of post-menopausal women, however, we found a more than 50% increased fracture risk for carriers of the variant studied over a 10 year period, implying that the GIPR variant may have a higher impact on bone metabolism than on glucose metabolism. There were no disturbances of lipid metabolism[38].

In conclusion, GIP has many possible actions and the GIPR is expressed in many tissues. Its function as an incretin hormone seems well established in humans, although lack of GIP effects only leads to minor disturbances of glucose metabolism. Its role in lipid metabolism is supported mainly by animal studies, whereas studies in humans have given ambiguous results. The concept of GIP as a regulator of bone metabolism is in its infancy, but the data so far support a role for GIP in the control of bone mass exerted by the gastrointestinal tract.

DISCLOSURES

The authors have no conflicts of interest in relation to this manuscript.

REFERENCES

1. Brown JC. Gastric inhibitory polypeptide. *Monogr Endocrinol* 1982; 24: III–XI, 1–88:III–88.
2. Holst JJ, Jensen SL, Knuhtsen S, *et al.* Effect of vagus, gastric inhibitory polypeptide, and HCl on gastrin and somatostatin release from perfused pig antrum. *Am J Physiol* 1983; 244: G515–G522.
3. Maxwell V, Shulkes A, Brown JC, *et al.* Effect of gastric inhibitory polypeptide on pentagastrin-stimulated acid secretion in man. *Dig Dis Sci* 1980; 25: 113–116.
4. Dupre J, Ross SA, Watson D, *et al.* Stimulation of insulin secretion by gastric inhibitory polypeptide in man. *J Clin Endocrinol Metab* 1973; 37: 826–828.
5. Krarup T. Immunoreactive gastric inhibitory polypeptide. *Endocr Rev* 1988; 9: 122–134.
6. Andersen DK, Elahi D, Brown JC, *et al.* Oral glucose augmentation of insulin secretion: interactions of gastric inhibitory polypeptide with ambient glucose and insuln levels. *J Clin Invest* 1978; 49: 152–161.
7. Vilsboll T, Krarup T, Madsbad S, *et al.* Both GLP-1 and GIP are insulinotropic at basal and postprandial glucose levels and contribute nearly equally to the incretin effect of a meal in healthy subjects. *Regul Pept* 2003; 114: 115–121.
8. Hojberg PV, Zander M, Vilsboll T, *et al.* Near normalisation of blood glucose improves the potentiating effect of GLP-1 on glucose-induced insulin secretion in patients with type 2 diabetes. *Diabetologia* 2008; 51: 632–640.
9. Vilsboll T, Knop FK, Krarup T, *et al.* The pathophysiology of diabetes involves a defective amplification of the late-phase insulin response to glucose by glucose-dependent insulinotropic polypeptide-regardless of etiology and phenotype. *J Clin Endocrinol Metab* 2003; 88: 4897–4903.
10. Christensen M, Vedtofte L, Holst JJ, *et al.* Glucose-dependent insulinotropic polypeptide: a bifunctional glucose-dependent regulator of glucagon and insulin secretion in humans. *Diabetes* 2011; 60: 3103–3109.
11. Gribble FM, Reimann F. Enteroendocrine cells: chemosensors in the intestinal epithelium. *Annu Rev Physiol* 2016; 78: 277–299.
12. Egerod KL, Engelstoft MS, Grunddal KV, *et al.* A major lineage of enteroendocrine cells coexpress CCK, secretin, GIP, GLP-1, PYY, and neurotensin but not somatostatin. *Endocrinology* 2012; 153: 5782–5795.
13. Svendsen B, Pedersen J, Jacob Wewer AN, *et al.* An analysis of co-secretion and co-expression of gut hormones from male rat proximal and distal small intestine. *Endocrinology* 2015; 156: 847–857.
14. Svendsen B, Pais R, Engelstoft MS, *et al.* GLP1 and GIP cells rarely overlap and differ by bombesin receptor-2 expression and responsiveness. *J Endocrinol* 2016; 228: 39–48.
15. Knop FK, Vilsboll T, Madsbad S, *et al.* Inappropriate suppression of glucagon during OGTT but not during isoglycaemic i.v. glucose infusion contributes to the reduced incretin effect in type 2 diabetes mellitus. *Diabetologia* 2007; 50: 797–805.
16. Lund A, Vilsboll T, Bagger JI, *et al.* The separate and combined impact of the intestinal hormones, GIP, GLP-1 and GLP-2, on glucagon secretion in type 2 diabetes. *Am J Physiol Endocrinol Metab* 2011; 300: E1038– E1046.
17. Lund A, Bagger JI, Christensen M, *et al.* Hyperglucagonemia after oral glucose and suppression of glucagon following intravenous glucose in totally pancreatectomized patients. *Diabetes* 2015; 64(Suppl. 1): A62–A63.
18. Bagger JI, Knop FK, Lund A, *et al.* Glucagon responses to increasing oral loads of glucose and corresponding isoglycaemic intravenous glucose infusions in patients with type 2 diabetes and healthy individuals. *Diabetologia* 2014; 57: 1720–1725.

19. Dirksen C, Bojsen-Moller KN, Jorgensen NB, *et al.* Exaggerated release and preserved insulinotropic action of glucagon-like peptide-1 underlie insulin hypersecretion in glucose-tolerant individuals after Roux-en-Y gastric bypass. *Diabetologia* 2013; 56: 2679–2687.
20. Yip RG, Wolfe MM. GIP biology and fat metabolism. *Life Sci* 2000; 66: 91–103.
21. Dawson JM, Greathead HM, Sessions VA, *et al.* Effect of gastric inhibitory polypeptide on bovine fat metabolism. *Comp Biochem Physiol B Biochem Mol Biol* 1999; 123: 79–88.
22. Miyawaki K, Yamada Y, Ban N, *et al.* Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med* 2002; 8: 738–742.
23. Ugleholdt R, Pedersen J, Bassi MR, *et al.* Transgenic rescue of adipocyte glucose-dependent insulinotropic polypeptide receptor expression restores high fat diet-induced body weight gain. *J Biol Chem* 2011; 286: 44632–44645.
24. Pedersen J, Ugleholdt RK, Jorgensen SM, *et al.* Glucose-metabolism is altered after loss of L- and alpha-cells, but not influenced by loss of K-cells. *Am J Physiol Endocrinol Metab* 2013; 304: E60–E73.
25. Asmar M, Tangaa W, Madsbad S, *et al.* On the role of glucose-dependent insulintropic polypeptide in postprandial metabolism in humans. *Am J Physiol Endocrinol Metab* 2010; 298: E614–E621.
26. Asmar M, Simonsen L, Madsbad S, *et al.* GIP may enhance fatty acid re-esterification in subcutaneous, abdominal adipose tissue in lean humans. *Diabetes* 2010; 59: 2160–2163.
27. Asmar M, Simonsen L, Arngrim N, *et al.* Glucose-dependent insulinotropic polypeptide has impaired effect on abdominal, subcutaneous adipose tissue metabolism in obese subjects. *Int J Obes (Lond)* 2014; 38: 259–265.
28. Moller CL, Vistisen D, Faerch K, *et al.* Glucose-dependent insulinotropic polypeptide (GIP) is associated with lower LDL but unhealthy fat distribution, independent of insulin: the ADDITION-PRO study. *J Clin Endocrinol Metab* 2016; 201: 485–493 jc20153133.
29. Bollag RJ, Zhong Q, Phillips P, *et al.* Osteoblast-derived cells express functional glucose-dependent insulinotropic peptide receptors. *Endocrinology* 2000; 141: 1228–1235.
30. Zhong Q, Itokawa T, Sridhar S, *et al.* Effects of glucose-dependent insulinotropic peptide on osteoclast function. *Am J Physiol Endocrinol Metab* 2007; 292: E543–E548.
31. Xie D, Cheng H, Hamrick M, *et al.* Glucose-dependent insulinotropic polypeptide receptor knockout mice have altered bone turnover. *Bone* 2005; 37: 759–769.
32. Morawska D, Sieklucka-Dziuba M, Kleinrok Z. Central action of glucagon. *Pol J Pharmacol* 1998; 50: 125–133.
33. Tsukiyama K, Yamada Y, Yamada C, *et al.* Gastric inhibitory polypeptide as an endogenous factor promoting new bone formation after food ingestion. *Mol Endocrinol* 2006; 20: 1644–1651.
34. Henriksen DB, Alexandersen P, Bjarnason NH, *et al.* Role of gastrointestinal hormones in postprandial reduction of bone resorption. *J Bone Miner Res* 2003; 18: 2180–2189.
35. Nissen A, Christensen M, Knop FK, *et al.* Glucose-dependent insulinotropic polypeptide inhibits bone resorption in humans. *J Clin Endocrinol Metab* 2014; 99: E2325–E2329.
36. Henriksen DB, Alexandersen P, Hartmann B, *et al.* Four-month treatment with GLP-2 significantly increases hip BMD: a randomized, placebo-controlled, dose-ranging study in postmenopausal women with low BMD. *Bone* 2009; 45: 833–842.
37. Henriksen DB, Alexandersen P, Byrjalsen I, *et al.* Reduction of nocturnal rise in bone resorption by subcutaneous GLP-2. *Bone* 2004; 34: 140–147.
38. Torekov SS, Harslof T, Rejnmark L, *et al.* A functional amino acid substitution in the glucose-dependent insulinotropic polypeptide receptor (GIPR) gene is associated with lower bone mineral density and increased fracture risk. *J Clin Endocrinol Metab* 2014; 99: E729–E733.
39. Saxena R, Hivert MF, Langenberg C, *et al.* Genetic variation in GIPR influences the glucose and insulin responses to an oral glucose challenge. *Nat Genet* 2010; 42: 142–148.