GIP as a Therapeutic Target in Diabetes and Obesity: Insight From Incretin Co-agonists

Jens Juul Holst1,2 and Mette Marie Rosenkilde1

1Department of Biomedical Sciences, University of Copenhagen, Copenhagen, Denmark; and 2NNF Center for Basic Metabolic Research, University of Copenhagen, Copenhagen, Denmark

ORCID numbers: 0000-0001-6853-3805 (J. J. Holst); 0000-0001-9600-3254 (M. M. Rosenkilde).

The 2 hormones responsible for the amplification of insulin secretion after oral as opposed to intravenous nutrient administration are the gut peptides, glucagon-like peptide-1 (GLP-1) and glucose-dependent insulinotropic polypeptide (GIP). However, whereas GLP-1 also inhibits appetite and food intake and improves glucose regulation in patients with type 2 diabetes (T2DM), GIP seems to be devoid of these activities, although the 2 hormones as well as their receptors are highly related. In fact, numerous studies have suggested that GIP may promote obesity. However, chimeric peptides, combining elements of both peptides and capable of activating both receptors, have recently been demonstrated to have remarkable weight-losing and glucose-lowering efficacy in obese individuals with T2DM. At the same time, antagonists of the GIP receptor have been reported to reduce weight gain/cause weight loss in experimental animals including nonhuman primates. This suggests that both agonists and antagonist of the GIP receptor should be useful, at least for weight-losing therapy. How is this possible? We here review recent experimental evidence that agonist-induced internalization of the two receptors differs markedly and that modifications of the ligand structures, as in co-agonists, profoundly influence these cellular processes and may explain that an antagonist may activate while an agonist may block receptor signaling.

Key Words: GLP-1, type 2 diabetes, weight-losing therapy, glucose-dependent insulinotropic polypeptide, receptor internalization, co-agonists.
2-digit percentages, with both effects exceeding what was obtained in the same study with a long-acting GLP-1 receptor agonist, dulaglutide. Increasing the confusion even further, another company, Amgen, presented preclinical data from nonhuman primates showing that a GIP receptor antagonist (a monoclonal antibody), both alone and in combination with GLP-1, effectively reduced the normal increase in body weight in obese animals (8). In addition, upon comparison of results from animal studies, it appears that almost identical results can be obtained with certain proven GIP agonists and GIP antagonists, at least with respect to their effects on body weight (8-10). In other words, both GIP agonism (normally thought to be inactive) and GIP antagonism appear to be effective in T2DM and obesity. How is this possible?

The full answer to this question cannot be given presently, but it appears that the manipulations with the agonist structure may have consequences that reach beyond the simple key-and-lock activation of its cognate receptor. This could happen if the manipulated co-agonist activates a different set of pathways compared to the endogenous agonists (also known as biased agonism). It should also be considered that a combined activation of the GIP as well as the GLP-1 system could result in beneficial effects beyond those obtained by a simple addition of the 2 separate effects, either by concomitant or by sequential activation of the 2 hormone systems.

GIP, a 42-amino acid polypeptide secreted from endocrine K-cells of the upper small intestinal epithelium, was the first incretin to be established (11,12), and in careful mimicry studies, Nauck et al (13) demonstrated that the insulinotropic effects of GIP infusions, resulting in plasma concentrations similar to those observed after oral glucose ingestion, could fully explain the insulin response to oral glucose. The insulinotropic effects of the other incretin hormone GLP-1 (14,15) and its possible incretin role (16), were described 1987. Again, in accurate mimicry studies, it was demonstrated that both GIP and GLP-1, infused to plasma concentrations precisely mimicking postprandial concentrations, would stimulate insulin secretion, about equally, in a glucose dependent manner; weakly at fasting glucose levels and more powerfully at higher (still normal) postprandial levels (17). Around 1992, Raufman and Eng in New York characterized the Gila monster peptide exenatide (18), which turned out to be a full agonist for the GLP-1 receptor (19) (a synthetic version of which, exenatide, reached the market in 2005 (20)). Building on experience from their isolation of the related peptide, exendin-3, they also identified a powerful and seemingly specific GLP-1 receptor antagonist, namely, the truncated peptide, exendin 9-39 (18). With this tool, which was administered to humans in 1998-1999 (21), it was possible to characterize the actions of endogenous GLP-1, and in several studies (22,23), the insulinotropic actions of endogenous GLP-1 were confirmed using exendin 9-39, which typically reduced insulin responses to oral/intestinal glucose administration in humans. For GIP, an effective and potent receptor antagonist for human use was only recently introduced (24), but with these tools at hand, it was now possible to analyze the combined actions of GIP and GLP-1 in the same experiment. Thus, in healthy subjects, infusions of each of the 2 antagonists reduced impaired oral glucose tolerance and reduced glucose induced insulin secretion. In combination, they clearly had additive effects (25). It was calculated that whereas glucose alone was responsible for 33% of the insulin response to oral glucose, GIP was responsible for 44% and GLP-1 for 22% of the response (26). While largely confirming the mimicry experiments, the use of exendin 9-39 is not unproblematic, because the antagonist also greatly increases plasma glucose and glucagon levels, which complicates the interpretation considerably (27).

As already alluded to, infusions of GLP-1 in supraphysiological amounts or administration of GLP-1 receptor agonists are capable of inducing insulin secretion and appetite reduction also in obese patients with T2DM, whereas infusions of GIP are remarkably ineffective, regardless of infusion rate (28). Also when infused together, only the GLP-1 part has apparent effects on insulin secretion and blood glucose. In fact, in an experiment with co-infusion, the suppression of glucagon secretion by GLP-1 was obliterated by co-infusion with GIP, perhaps in agreement with the observation that GIP, if anything, stimulates glucagon secretion, particularly in T2DM patients, an effect that might actually contribute to the development of hyperglycemia (29). In further studies of appetite and food intake, infusions of GLP-1 increased insulin secretion and inhibited food intake, whereas infusions of pharmacological amounts of GIP were ineffective and even appeared to prevent the inhibitory effects of GLP-1 on food intake (30). A similar finding was made in experiments in which GLP-1 agonism had been maintained chronically, namely, in patients with T2DM during stable therapy with the GLP-1 RA, liraglutide. In these patients, infusions of pharmacological amounts of GIP increased glucagon concentrations, impaired postprandial lipids, and increased postprandial glycaemia but had no effect on food intake (31). In view of these studies and many more, which all
consistently have demonstrated lack of GIP efficacy in T2DM, the results obtained with the new GIP/GLP-1 co-agonists seem incomprehensible.

The inactivity of GIP in T2DM has been investigated in experimental animal models, and it has been reported that hyperglycemia reduces GIP receptor expression in the beta cells and that treatment of the hyperglycemia restores GIP receptor expression and beta cell responsiveness (32). In studies of individuals with long-standing T2DM, in whom physiological GIP infusions were completely without effect, a 4-week period of intensive, basal-bolus insulin therapy, which nearly normalized glucose levels, there was some restoration of GIP’s insulinotropic effect, but the responses were still very far from normal levels or levels observed after pharmacological GLP-1 therapy (33). In addition, during clamp studies in patients with T2DM (28), a small early insulin response may actually be induced with high dose GIP infusions (whereas the second-phase response is completely absent), and this early response is, although smaller than that observed in controls, impaired to the same extent as the early response to GLP-1. This suggests that the overall loss of the insulinotropic of GIP in T2DM is due to postreceptor defects associated with prolonged beta cell stimulation, rather than to a specific, glucose-induced down-regulation of the GIP receptor (34).

Upon further analysis of co-administrations of the GLP-1 and GIP antagonists under various circumstances, additional interesting features have emerged. In healthy individuals, GIP has effects other than stimulating insulin secretion. GIP is probably one of the important actors in the so-called gut-bone axis, a term introduced to describe the 50% reduction in bone resorption (as measured by bone resorption markers; eg, C-terminal telopeptide of type 1 collagen) that occurs after food intake, compared to the fasting rate (35). Thus, during most of the daytime, bone resorption is reduced, while a corresponding increase is observed during the night time, whereby a constant bone mass is maintained. It turns out that GIP infusions in humans are capable of causing similar reductions in bone resorption (36). The effect is apparently due to a direct effect of the hormone on GIP receptors expressed on both osteoblasts and osteoclasts, the functions of which are, respectively, enhanced and inhibited. In agreement with the supposed actions of GIP, administration of the GIP antagonist GIP (3–29) \( \text{NH}_2 \) greatly reduced the meal-induced suppression of bone resorption, and these experiments thus confirmed the important contribution of GIP to the gut-bone axis (37). In further studies, it turned out that administration of the same GIP antagonist markedly reduced the meal-induced bone resorption, even in individuals with T2DM. First, this indicates that the GIP part of the gut-bone axis is also operative in these patients, and second, it suggests that GIP receptor expression and function in the bone cells is not affected in T2DM (38). It can therefore be concluded that if a change in GIP receptor expression or function is involved in the impaired insulin response to GIP in T2DM, this change is likely to be relevant only for GIP receptors expressed in beta cells.

The studies supporting antidiabetic and weight-reducing actions of GIP and GIP co-agonists date back to an early study in rodents with a monomolecular GIP-GLP-1 co-agonist, which was found to both enhance glucose tolerance and to lower body weight (39). This was of cause unexpected since GIP in humans, as previously discussed, exerted opposite effects in combinations with GLP-1 infusions. In fact, GIP had for a long time and for many reasons been considered “the obesity hormone” (40); for instance, its secretion is enhanced by intake of fatty meals, and GIP infusions in experimental animals were reported to enhance chylomicron clearance and fat deposition (41). Indeed, in 2002, mice with a knockout of the GIP receptor were demonstrated to be resistant to the adipogenic effect of a high-fat diet (42), and human genetic studies identified inactivating (Rosenkilde et al, unpublished) mutations in the GIP receptor, which were associated with weight loss (43). Altogether, rather than promoting weight loss, it was anticipated that GIP actions would promote weight gain and that a rational approach to obesity therapy therefore might be application of a GIP antagonist. Indeed, GIP antagonism in the form of a monoclonal antibody against the GIP receptor turned out to be effective with respect to inhibiting food intake and promoting a weight loss in both rodents and in obese nonhuman primates (8). However, what was clearly missing in the human studies was a long-acting GIP antagonist, and there are still no data available regarding long-term actions of GIP agonism in humans. In rodents, however, long-acting GIP agonists with an improved design were recently reported to have in weight losing properties (44), and in the same series of studies long-acting (acylated) GIP agonists did not cause weight loss in diet-induced obese animals. Furthermore, recent elegant studies suggested that certain somatostatinergic neurons in the rodent hypothalamus express GIP receptors and react to activation of these by decreasing food intake (45). These newer findings raise the question whether there are species differences regarding the effects of GIP on appetite and food intake.

Currently, therefore, we have two opposing viewpoints, one maintaining that GIP antagonism would be beneficial with respect to at least weight management
and the other proposing that GIP agonism, perhaps preferably in conjunction with GLP-1 agonism, would be effective.

Is It at All Possible to Reconcile the Two Viewpoints?

The people behind the development of the GIP receptor antibody have looked at the possible mechanisms (10) and focused on GIP receptor down regulation. It is known that GIP activation of its receptor is associated with recruitment of beta arrestins and that arrestins are needed for the subsequent internalization of the hormone receptor complex (46). By extended exposure of a GIP receptor expressing tissue to GIP, it would therefore be possible to create profound down regulation and therefore desensitization of the GIP receptor and impairment of the GIP sensitivity of the tissue. Indeed, this was directly demonstrated by Mohammad et al (47), who showed that an initial GIP stimulation can impair subsequent GIP stimulations, associated with disappearance of GIPR from the plasma membrane in 3T3-L1 adipocytes. This mechanism would be consistent with the remarkable lack of responses to increasing GIP concentrations, brought about by infusions of GIP, on top of the normal meal responses in healthy subjects (6). Furthermore, it was recently shown that the GIP receptor antagonist GIP (3–29) NH2 was able to restore the cell surface expression of the GIP receptor in transfected HEK293 cells after pre-incubation (and thereby agonist-induced receptor internalization) with endogenous GIP (46). Hence, it may be anticipated that antagonizing endogenous GIP actions in vivo, as can be done with both receptor antibodies and with peptide-based GIP receptor antagonists including GIP (3-29)NH2 in humans, would result in increased receptor expression on the cell surface, whereby the sensitivity of the system is regained. It is, however, still difficult to understand how GIP can activate the receptor in the presence of an antagonist, given the competitive nature of at least peptide-based GIPR antagonists (48). Nevertheless, the receptor internalization process is apparently important for GIP actions. For instance, when studied in vitro, the well-known GIP receptor mutation E354Q, which is associated with impaired glucose tolerance and increased fracture risk in postmenopausal women (49), actually shows enhanced agonist-mediated and basal 3',5'-cyclic AMP formation and maintained arrestin recruitment, but prolonged agonist residence time, resulting in accelerated internalization and therefore impaired overall activation of the receptor signaling (50,51). This mutation is also associated with a slower recycling of internalized receptors to the cell surface, which, although it has been shown that the GIP receptor may also signal from endosomes (52), probably contributes to an overall impaired receptor function.

Thus, an effect on receptor recycling is apparently important for the actions of both GIP agonists and antagonists. But what about the effects of the GIP-GLP-1 co-agonists and their apparently beneficial metabolic actions? As previously discussed, the beneficial effect of GIP receptor activation is difficult to understand, as the effect of GIP is impaired in patients suffering from T2DM and obesity. So how can a dual-acting GIP-GLP-1 receptor agonist be better than the GLP-1 part of the combination? At first, it might be considered whether this is indeed the case. Upon closer scrutiny, the first dual GIP-GLP-1 co-agonist (NN9709, formerly MAR709 and RG7697) wasn’t terribly impressive after all, and its performance in a Phase 2 clinical trial did not differ from that of liraglutide (53). The second, tirzepatide, was clearly superior to the GLP-1 RA control, dulaglutide, in the dose-finding Phase 2 study mentioned in the beginning (7) although it was not ensured that optimal dosing had been investigated for the comparator—the fact that increasing doses of dulaglutide are currently being investigated (54) might suggest that the dose employed in the Phase 2 study was suboptimal. Nevertheless, as already mentioned, it is possible that the administration of a molecule that can activate both the GIP and the GLP-1 receptor may be beneficial in a sequential manner. Thus, the activation of the GLP-1 system might be the primary beneficial action, so that the beneficial effect of GIP may only be observed after metabolic control has been (partly) restored by GLP-1. In other words, the insulinotropic action of GIP may be regained after a GLP-1-mediated lowering of the blood glucose in agreement with the beneficial effects of intensive insulin therapy as previously mentioned (33). However, the disappointing results of adding high-dose GIP infusions to chronic liraglutide treatment (31) speak against this possibility. Another explanation could lie in a different pharmacodynamic profile of the dual agonist as compared to the individual signaling profiles of GIP and GLP-1, for instance caused by altered signaling of 1 or both of the 2 components. In fact, it has been shown that even small changes in the GIP as well as the GLP-1 molecule may change the receptor signaling towards a preferential G protein signaling with decreased arrestin recruitment and/or reduced receptor internalization (for GIP changes, see (51); for GLP-1 changes, (55, 56)). For the GIP system, such an effect would be beneficial due to a lower degree of receptor desensitization and internalization, and thereby improved therapeutic effect, given the proven downregulation of this
system upon prolonged GIP administration (47,51,50). For the GLP1-1 system, receptor internalization seems independent of arrestin recruitment (57). Nevertheless, it was recently shown that N-terminal modifications of exendin-4 (a strong GLP-1 receptor agonist) will turn it into a biased agonist with a lower tendency to arrestin recruitment and/or receptor internalization and therefore with potentially greater efficacy and tolerability as a therapeutic (58). A similar alteration in GLP-1 receptor signaling profile was recently established for a dual acting GIP-GLP-1 peptide (59). It is thus possible that the observed beneficial effects in vivo of dual GIP-GLP-1 agonists—at least partly—rely on altered signaling of the molecule toward a biased signaling profile for one, or both, of the components. If both mechanisms apply to the GIP- GLP-1 co-agonists, the effect might be even greater. It is still unclear how the GIP part of the co-agonist would lead to weight loss, but if the incorporation of GIP activity in the co-agonist changes the GLP-1 signaling, then it would make sense that even the GIP part of the molecule might contribute to an enhanced weight-losing effect. It should be possible with careful molecular pharmacological experimentation to determine whether it is the influence of one part of the co-agonist (GIP) on the signaling pathways of the other part that makes a co-agonist like tirzepatide so effective, despite the overwhelming evidence that GIP, investigated in isolation, does not possess these activities. Such experiments are ongoing, and we will probably soon have at least some answers to this mind-boggling paradox.

Additional Information

Correspondence and Reprint Requests: Jens Juul Holst, Department of Biomedical Sciences, the Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen, Denmark. E-mail: jjholst@sund.ku.dk. Mette Marie Rosenkilde, Department of Biomedical Sciences, the Panum Institute, Blegdamsvej 3, DK-2200 Copenhagen, Denmark. E-mail: Rosenkilde@sund.ku.dk.

Disclosure Summary: JJH and MMR are co-founders of Antag Therapeutics and Beinan Biotech. JJH serves on advisory boards for NovoNordisk.

Data Availability: Data sharing is not applicable to this article as no datasets were generated or analyzed during the current study.

References

1. Nauck MA, Homberger E, Siegel EG, et al. Incretin effects of increasing glucose loads in man calculated from venous insulin and C-peptide responses. J Clin Endocrinol Metab. 1986;63(2):492-498.
2. Nauck M, Stockmann F, Ebert R, Creutzfeldt W. Reduced incretin effect in type 2 (non-insulin-dependent) diabetes. Diabetologia. 1986;29(1):46-52.
3. Knop FK, Vilsholl T, Hojberg PV, et al. Reduced incretin effect in type 2 diabetes: cause or consequence of the diabetic state? Diabetes. 2007;56(8):1951-1959.
4. Nauck MA, Heimesaat MM, Orskov C, Holst JJ, Ebert R, Creutzfeldt W. Preserved incretin activity of glucagon-like peptide 1 [7-36 amide] but not of synthetic human gastric inhibitory polypeptide in patients with type-2 diabetes mellitus. J Clin Invest. 1993;91(1):301-307.
5. Flint A, Raben A, Astrup A, Holst JJ. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. J Clin Invest. 1998;101(3):515-520.
6. Asmara M, Tangaw W, Madshad S, et al. On the role of glucose-dependent insulintropic polypeptide in postprandial metabolism in humans. Am J Physiol Endocrinol Metab. 2010;298(3):E614-E621.
7. Frier JP, Nauck MA, Van J, et al. Efficacy and safety of LY3298176, a novel dual GIP and GLP-1 receptor agonist, in patients with type 2 diabetes: a randomised, placebo-controlled and active comparator-controlled phase 2 trial. Lancet. 2018;392(10160):2180-2193.
8. Kilián EA, Wang J, Ye J, et al. Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models. Sci Transl Med. 2018;10(472):eaat3392.
9. Norregaard PK, Deryabina MA, Tofteng Shelton P, et al. A novel GIP analogue, ZP4165, enhances glucagon-like peptide-1-induced body weight loss and improves glycemic control in rodents. Diabetes Obes Metab. 2018;20(1):60-68.
10. Kilián EA, Lu SC, Fort M, Yamada Y, Veniant MM, Lloyd DJ. Glucose-dependent insulintropic polypeptide receptor therapies for the treatment of obesity, do agonists = antagonists? Endocr Rev. 2020;41(1):bnz002.
11. Brown JC. Gastric inhibitory polypeptide. Monogr Endocrinol. 1982;24(3-11):1-88.
12. Dupre J, Ross SA, Watson D, Brown JC. Stimulation of insulin secretion by gastric inhibitory polypeptide in man. J Clin Endocrinol Metab. 1973;37(5):826-828.
13. Nauck M, Schmidt WE, Ebert R, et al. Insulintropic properties of synthetic human gastric inhibitory polypeptide in man: interactions with glucose, phenylalanine, and cholecystokinin-8. J Clin Endocrinol Metab. 1989;69(3):654-662.
14. Holst JJ, Orskov C, Nielsen OW, Schwartz TW. Truncated glucagon-like peptide I, an insulin-releasing hormone from the distal gut. FEBS Lett. 1987;211(2):169-174.
15. Moosov S, Weir GC, Habener JF. Insulintropic glucagon-like peptide I (7-37) co-encoded in the glucagon gene is a potent stimulator of insulin release in the perfused rat pancreas. J Clin Invest. 1987;79(2):616-619.
16. Kreymann B, Williams G, Ghatari MA, Bloom SR. Glucagon-like peptide-1 7-36: a physiological incretin in man. Lancet. 1987;2(8571):1300-1304.
17. Vilsholl T, Krarup T, Madshad S, Holst JJ. Both glucagon-like peptide-1 (GLP-1) and glucosedependent insulintropic polypeptide (GIP) are insulintropic at basal and postprandial glucose levels in healthy subjects. Regul Pept. 2003;114:115-121.
18. Eng J, Kleinman WA, Singh L, Singh G, Raufman JP. Isolation and characterization of exendin-4, an exendin-3 analogue, from Heloderma suspectum venom. Further evidence for an exendin receptor on dispersed acini from guinea pig pancreas. J Biol Chem. 1992;267(11):7402-7405.
19. Raufman JP, Singh L, Singh G, Eng J. Truncated glucagon-like peptide-1 interacts with exendin receptors on dispersed acini from guinea pig pancreas. Identification of a mammalian analogue of the reptilian peptide exendin-4. J Biol Chem. 1992;267(30):21432-21437.
20. Buse JB, Henry RR, Han J, Kim DD, Fineman MS, Baron AD; Exenatide-113 Clinical Study Group. Effects of exenatide (exendin-4) on glycemic control over 30 weeks in sulfonylurea-treated patients with type 2 diabetes. *Diabetes Care.* 2004;27(11):2628-2635.

21. Edwards CM, Todd JE, Mahmoudi M, et al. Glucagon-like peptide-1 has a physiological role in the control of postprandial glucose in humans; studies with the antagonist exenin 9-39. *Diabetes.* 1999;48(1):86-93.

22. Schirra J, Sturm K, Leicht P, Arnold R, Göke B, Katschinski M. Exendin(9-39)amide is an antagonist of glucagon-like peptide-1(7-36)amide in humans. *J Clin Invest.* 1998;101(7):1421-1430.

23. Salehi M, Vahl TR, D’Alessio DA. Regulation of islet hormone release and gastric emptying by endogenous glucagon-like peptide 1 after glucose ingestion. *J Clin Endocrinol Metab.* 2008;93(12):4909-4916.

24. Hansen LS, Sparre-Ulrich AH, Christensen M, et al. N-terminally and C-terminally truncated forms of glucose-dependent insulinotropic polypeptide are high-affinity competitive antagonists of the human GIP receptor. *Br J Pharmacol.* 2016;173(5):826-838.

25. Gasbjerg LS, Helsted MM, Hartmann B, et al. Separate and combined glucolipemic effects of endogenous glucose-dependent insulinotropic polypeptide and glucagon-like peptide 1 in healthy individuals. *Diabetes.* 2019;68(5):906-917.

26. Nauck MA, Meier JJ. GIP and GLP-1: stepsiblings rather than monozygotic twins within the incretin family. *Diabetes.* 2019;68(5):897-900.

27. Gasbjerg LS, Bergmann NC, Stensen S, et al. Evaluation of the incretin effect in humans using GIP and GLP-1 receptor antagonists. *Peptides.* 2020;125:170183.

28. Vilsbøll T, Krarup T, Madsbad S, Holst JJ. Defective amplification of the late phase insulin response to glucose by GIP in obese Type II diabetic patients. *Diabetologia.* 2002;45(8):1111-1119.

29. Mentis N, Vardarli I, Köthe LD, et al. GIP does not potentiate the antidiabetic effects of GLP-1 in hyperglycemic patients with type 2 diabetes. *Diabetes.* 2011;60(4):1270-1276.

30. Bergmann NC, Lund A, Gasbjerg LS, et al. Effects of combined GIP and GLP-1 infusion on energy intake, appetite and energy expenditure in overweight/obese individuals: a randomised, crossover study. *Diabetologia.* 2019;62(4):665-675.

31. Bergmann NC, Gasbjerg LS, Heimbürger SM, et al. No acute effects of exogenous glucose-dependent insulinotropic polypeptide on energy intake, appetite, or energy expenditure when added to treatment with a long-acting glucagon-like peptide 1 receptor agonist in men with type 2 diabetes. *Diabetes Care.* 2020;43(3):588-596.

32. Piteau S, Olver A, Kim SJ, et al. Reversal of islet GIP receptor down-regulation and resistance to GIP by reducing hyperglycemia in the Zucker rat. *Biochem Biophys Res Commun.* 2007;362(4):1007-1012.

33. Højbjerg PV, Zander M, Vilsbøll 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(4):632-640.

34. Meier JJ, Gallwitz B, Kask B, et al. Stimulation of insulin secretion by intravenous bolus injection and continuous infusion of gastric inhibitory polypeptide in patients with type 2 diabetes and healthy control subjects. *Diabetes.* 2004;53(Suppl 3):S220-S224.

35. Bjørnason NH, Henriksen EE, Alexandersen P, Christgau S, Henriksen DB, Christiansen C. Mechanism of circadian variation in bone resorption. *Bone.* 2002;30(1):307-313.

36. Nissen A, Christensen M, Knop FK, Vilsbøll T, Holst JJ, Hartmann B. Glucose-dependent insulinotropic polypeptide inhibits bone resorption in humans. *J Clin Endocrinol Metab.* 2014;99(11):E2325-E2329.

37. Gasbjerg LS, Hartmann B, Christensen MB, et al. GIP’s effect on bone metabolism is reduced by the selective GIP receptor antagonist GIP(3-30)NH2. *Bone.* 2020;130:115079.

38. Stensen S, Gasbjerg LS, Krogh LL, et al. Endogenous glucose-dependent insulinotropic polypeptide exerts diverging and tissue specific effects in obese patients with type 2 diabetes. *Diabetologia.* 2019;62:S270-S271.

39. Finan B, Ma T, Ottaway N, et al. Unimolecular dual incretins maximize metabolic benefits in rodents, monkeys, and humans. *Sci Transl Med.* 2013;5(209):209ra151.

40. Marks V. GIP: the obesity hormone. In: James WPT, Parker SW, eds. *Current Approaches: Obesity.* Southampton, England: Duphar Medical Relations; 1988:13-19.

41. Yip RG, Wolfe MM. GIP biology and fat metabolism. *Life Sci.* 2000;66(2):91-103.

42. Miyawaki K, Yamada Y, Ban N, et al. Inhibition of gastric inhibitory polypeptide signaling prevents obesity. *Nat Med.* 2002;8(7):738-742.

43. Turcott V, Lu Y, Highland HM, et al.; CHD Exome+ Consortium; EPIC-CVD Consortium; ExomeBP Consortium; Global Lipids Genetic Consortium; GoT2D Genes Consortium; EPIC InterAct Consortium; INTERVAL Study; ReproGen Consortium; T2D-Genes Consortium; MAGIC Investigators; Understanding Society Scientific Group. Protein-altering variants associated with body mass index implicate pathways that control energy intake and expenditure in obesity. *Nat Genet.* 2018;50(1):26-41.

44. Mroz PA, Finan B, Gelfanov V, et al. Optimized GIP analogs promote body weight lowering in mice through GIPR agonism not antagonism. *Mol Metab.* 2019;20:51-62.

45. Adriaensen AE, Biggs EK, Darwish T, et al. Glucose-dependent insulinotropic polypeptide receptor-expressing cells in the hypothalamus regulate food intake. *Cell Metab.* 2019;30(5):987-996.e6.

46. Gabe MBN, Sparre-Ulrich AH, Pedersen MF, et al. Human GIP(3-30)NH2 inhibits G protein-dependent as well as G protein-independent signaling and is selective for the GIP receptor with high-affinity binding to primate but not rodent GIP receptors. *Biochem Pharmacol.* 2018;150:97-107.

47. Mohammad S, Patel RT, Bruno J, Panhwar MS, Wen J, McGraw TE. A naturally occurring GIP receptor variant undergos enhanced agonist-induced desensitization, which impairs GIP control of adipose insulin sensitivity. *Mol Cell Biol.* 2014;34(19):3618-3629.

48. Sparre-Ulrich AH, Gabe MN, Gasbjerg LS, et al. GIP(3-30)NH2 is a potent competitive antagonist of the GIP receptor and effectively inhibits GIP-mediated insulin, glucagon, and somatostatin release. *Biochem Pharmacol.* 2017;131:78-88.

49. Torekov SS, Ma L, Grarup N, et al.; GIANT Consortium. Homozygous carriers of the G allele of rs4664447 of the glucagon gene (GCG) are characterised by decreased fasting and stimulated levels of insulin, glucagon and glucagon-like peptide (GLP)-1. *Diabetologia.* 2011;54(11):2820-2831.

50. Gabe MBN, van der Velden WJC, Gadgaard S, et al. Enhanced agonist residence time, internalization rate and signalling of the GIP receptor variant [E354Q] facilitate receptor desensitization and long-term impairment of the GIP system. *Basic Clin Pharmacol Toxicol.* 2019.

51. Ismail S, Dubois-Vedrenne I, Laval M, et al. Internalization and desensitization of the human glucose-dependent-insulinotropic receptor is affected by N-terminal acetylation of the agonist. *Mol Cell Endocrinol.* 2015;414:202-215.

52. Ismail S, Gherardi MJ, Froese A, et al. Internalized receptor for glucose-dependent-insulinotropic peptide stimulates adenylylcyclase on early endosomes. *Biochem Pharmacol.* 2016;120:33-45.

53. Frias JP, Bastry EJ 3rd, Vignati L, et al. The sustained effects of a dual GIP/GLP-1 receptor agonist, NNCC090-2746, in patients with type 2 diabetes. *Cell Metab.* 2017;26(2):343-352.e2.

54. Frias JP, Wynne AG, Matyjaszek-Matuszek B, et al. Efficacy and safety of an expanded dulaglutide dose range: a phase 2,
placebo-controlled trial in patients with type 2 diabetes using metformin. *Diabetes Obes Metab.* 2019;21(9):2048-2057.

55. Hager MV, Clydesdale L, Gellman SH, Sexton PM, Wooten D. Characterization of signal bias at the GLP-1 receptor induced by backbone modification of GLP-1. *Biochem Pharmacol.* 2017;136:99-108.

56. Fremaux J, Venin C, Mauran L, et al. Ureidopeptide GLP-1 analogues with prolonged activity in vivo via signal bias and altered receptor trafficking. *Chem Sci.* 2019;10(42):9872-9879.

57. Syme CA, Zhang L, Bisello A. Caveolin-1 regulates cellular trafficking and function of the glucagon-like Peptide 1 receptor. *Mol Endocrinol.* 2006;20(12):3400-3411.

58. Jones B, Buenaventura T, Kanda N, et al. Targeting GLP-1 receptor trafficking to improve agonist efficacy. *Nat Commun.* 2018;9(1):1602.

59. Al-Zamel N, Al-Sabah S, Luqmani Y, et al. A dual GLP-1/GIP receptor agonist does not antagonize glucagon at its receptor but may act as a biased agonist at the GLP-1 receptor. *Int J Mol Sci.* 2019;20(14):3532.