Review

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Targeting the GIPR for obesity: To agonize or antagonize? Potential mechanisms

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ABSTRACT

Background: Glucose-dependent insulinotropic peptide (GIP) is one of two incretin hormones that communicate nutrient intake with systemic metabolism. Although GIP was the first incretin hormone to be discovered, the understanding of GIP's biology was quickly outpaced by research focusing on the other incretin hormone, glucagon-like peptide 1 (GLP-1). Early work on GIP produced the theory that GIP is obesogenic, limiting interest in developing GIPR agonists to treat type 2 diabetes. A resurgence of GIP research has occurred in the last five years, reinvigorating interest in this peptide. Two independent approaches have emerged for treating obesity, one promoting GIPR agonism and the other antagonism. In this report, evidence supporting both cases is discussed and hypotheses are presented to reconcile this apparent paradox.

Scope of the review: This review presents evidence to support targeting GIPR to reduce obesity. Most of the focus is on the effect of singly targeting the GIPR using both a gain- and loss-of-function approach, with additional sections that discuss co-targeting of the GIPR and GLP-1R.

Major conclusions: There is substantial evidence to support that GIPR agonism and antagonism can positively impact body weight. The long-standing theory that GIP drives weight gain is exclusively derived from loss-of-function studies, with no evidence to support that GIPR agonisms increases adiposity or body weight. There is insufficient evidence to reconcile the paradoxical observations that both GIPR agonism and antagonism can reduce body weight; however, two independent hypotheses centered on GIPR antagonism are presented based on new data in an effort to address this question. The first discusses the compensatory relationship between incretin receptors and how antagonism of the GIPR may enhance GLP-1R activity. The second discusses how chronic GIPR agonism may produce desensitization and ultimately loss of GIPR activity that mimics antagonism. Overall, it is clear that a deeper understanding of GIP biology is required to understand how modulating this system impacts metabolic homeostasis.

Keywords Incretins; Obesity; Type 2 diabetes; Glucose-dependent insulinotropic polypeptide (GIP)

1. INTRODUCTION

1.1. The incretin system

The incretin axis originates with the activation of enteroendocrine cells in the intestine in response to food intake. The principle incretins are glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide 1 (GLP-1) produced by K cells in the proximal gut and L cells in the distal gut, respectively [1]. The most well-documented actions of incretins are the stimulation of insulin secretion in β cells through activation of the GIP receptor (GIPR) and GLP-1 receptor (GLP-1R), but incretins also regulate gastric motility, nutrient absorption, blood flow, and food intake [1]. Because of these actions, the incretin axis serves as a proximal step in the communication of food intake to the systems that regulate postprandial metabolic homeostasis, a concept that is reinforced by reports that interruptions in the incretin axis cause glucose intolerance [2–4]. Importantly, research on incretin physiology has produced two classes of antidiabetic drugs: i) GLP-1R agonists and ii) inhibitors of dipeptidyl peptidase 4 (DPP4), the enzyme that cleaves and inactivates both incretin peptides [5]. GLP-1R agonists are now used for body weight lowering in non-diabetic obese patients [6] and serve as the foundation for multireceptor agonists that are being developed to treat diabetes, obesity, and related comorbidities [7].

The clinical success of GLP-1R agonists in treating type 2 diabetes (T2D) has produced significant scientific interest in GLP-1 for the past two decades, research that has unraveled novel mechanisms to explain the glucose control and weight-lowering properties of these agonists and produce additional potential indications including cardiovascular and renal benefits. However, the spotlight on GLP-1 had an unintended effect to diminish interest in GIP over this same time period. Indeed, GIPR agonism for treating T2D was largely dismissed because of reports that: 1) GIPR agonists fail to stimulate insulin secretion in people with T2D, even at pharmacological levels [8]; 2) GIP stimulates glucagon secretion, which exacerbates hyperglycemia in T2D [9]; and 3) GIP is obesogenic through direct actions in adipose tissue [10]. The potential contribution of GIP to obesity has been reinforced by human genome-wide association studies (GWAS), which have identified *GIPR* as a gene that contributes to body mass index (BMI) [11–13]. One study reported that variants in the *GIPR* locus associated with elevated glycemia, decreased insulin secretion, and a diminished incretin response suggested loss of function in the GIPR

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[12]. This same variant was associated with reduced BMI, reinforcing the preclinical data in *Gipr* knockout mice ([10] and discussed in detail to follow) that reports that loss of GIPR signaling protects against diet-induced obesity.

A GIP renaissance has recently emerged and many previously held ideas have been challenged [14], forcing a reconsideration of the historical data that shaped the consensus view on what had become the lesser incretin. GIPR agonism is being pursued as an anti-hyperglycemic therapy for treating T2D, frequently in combination with GIPR agonism with GLP-1R activity [7,15]. Furthermore, glucagon is no longer viewed exclusively as a hyperglycemic agent, as the insulinotropic actions of glucagon on β cells can lower glycemia [16–18]. In light of this, GIPR activity in α cells has been shown to contribute to the incretin axis by inducing greater insulin secretion through α to β cell communication facilitated by glucagon secretion than that achieved by GIPR activity in β cells alone [19]. While some of the early theories of GIP biology are being reformed, GIP activity's contribution to body weight remains debated. Indeed, there is evidence mounting on both sides of this debate that provides compelling arguments that both GIPR agonism and antagonism can reduce adiposity and body weight. This review examines the evidence that explores the mechanisms by which GIPR activity regulates energy homeostasis and ultimately body weight in an attempt to reconcile how both GIPR agonists and antagonists can improve metabolic outcomes in diabetes and obesity.

1.2. GIPR tissue expression

The GIPR is expressed in select cell types throughout the body, many of which exert direct or indirect control over body weight [20]. There is a consensus for many of the reported GIPR + tissues, supported by detailed gene expression analysis and functional data. However, some tissues reported to be GIPR + are questionable, with mixed degrees of support. Some of the difficulty in assessing whether a particular cell type expresses a functional receptor is the lack of quality reagents needed to ascertain the expression of GIPR. Examples of such tools have been generated for the GLP-1R, including validated antibodies [21–23], modified ligands that permit receptor labeling [24], effective and well-established antagonists [25,26], or reporter mouse models [27,28]. The generation of these tools for the GIPR has lagged and powerful research tools have only recently become available [29]. Ultimately, reagents that target GIPR protein at the cell surface combined with functional studies using validated gain- and loss-of-function tools remain the gold standard to ascertain the potential activity of GIP in a given cell type. Much of the current information is based on gene expression and RNA levels, which can be misleading for class B GPCRs such as the GIPR or GLP-1R [22]. This section discusses the tissue expression patterns of GIPR to highlight the potential locations where modulation of GIP activity can impact body weight or glucose metabolism.

1.2.1. Islets

Pancreatic islets robustly express the GIPR [30], and this tissue is commonly used as a positive control for expression levels [29,31]. RNA analysis of mouse islets shows similar expression levels among α, β, and δ cells [32], aligning with reports that GIPR agonists increase glucagon [8,33], insulin [30], and somatostatin [34] secretion in rodents and humans. Single-cell RNA sequencing (scRNAseq) of human islets also supports *GIPR* expression in α, β, and δ cells along with similar expression levels in γ cells [35].

1.2.2. Adipose tissue

The GIPR has been identified in multiple adipose tissue depots. *Gipr/GIPR* levels are detectable in rodent [36] and human [37] white adipose tissue (WAT) samples, but the cellular source of this signal among heterogenous populations of cells within this tissue is unclear. Much of the literature investigating the role of GIPR in "white adipocytes" was derived from rodent [38] and human [39] differentiated cell lines originating from progenitor cells, for example, 3T3-L1 cells. The expression of *Gipr/GIPR* is absent in precursor cells and robustly increases upon chemical induction of differentiation. The extent to which this in vitro process replicates the expression levels of primary white adipocytes is known. Cre-mediated deletion of *Gipr* specifically in adipose tissue via the *Ap2* promoter reduced expression in both visceral and subcutaneous adipose depots [40], supporting the expression of *Gipr* in adipocytes. However, there has been some skepticism of this result from reports that *Ap2* expression is "leaky" and enables Cre activity in endothelial cells, macrophages, adipocyte precursors, and the brain [41]. *Gipr* expression was reported in endothelial cells [42–44] and adipose tissue macrophages [45,46], although the relative levels compared to adipocytes and the tissue specificity of these cellular sources remains unclear. Transgenic expression of Cre under control of the *Gipr* promoter produced reporter activity in some but not all adipocytes [29], further highlighting the complexity of the cellular source of GIPR expression in WAT. Analysis of *Gipr* expression in rodent brown adipose tissue (BAT) has produced clearer outcomes. RNA levels of *Gipr* are detectable in mouse BAT and using *Myf5-Cre* to specifically target BAT effectively eliminates *Gipr* levels in BAT but not WAT [47]. Expression of *GIPR* in human brown or beige tissue has not been specifically investigated.

1.2.3. Brain

There is widespread expression of *Gipr* in the rodent brain. *Gipr* expression has been reported in the cerebral cortex, hippocampus, olfactory bulb, brain stem, and cerebellum in rats [20], which aligns with regions identified by radiolabeled GIP-binding assays [48], in situ hybridization [49], and qPCR analysis [50]. The development of GIPR reporter mice, generated by crossing mice expressing Cre recombinase under control of the endogenous *Gipr* promoter with YFP reporter mice (GIPR-YFP), provided higher resolution into the various subregions within the brain and potential characteristics of GIPR + neurons [29]. *Gipr* activity was confirmed in the olfactory bulb, cortex, and hippocampal regions and also reported in additional regions including the medial preoptic area, subfornical organ, anterodorsal thalamic nucleus, paraventricular thalamic nucleus, magnocellular preoptic nucleus, suprachiasmatic nucleus, and interfascicular nucleus. *Gipr* was also found in the paraventricular, dorsomedial, and arcuate nuclei of the hypothalamus. Single-cell RNA sequencing (scRNAseq) in *Gipr*-positive neurons in the hypothalamus produce co-expression with the neurohormones *Sst*, *Avp*, *Tac1*, and *Cartpt*, peptides previously implicated in energy homeostasis. Characterizing the genes that could contribute to the cell surface receptor profile of *Gipr* neurons provides clues to the potential regulation of these neurons, including receptors for glutamate, GABA, opioids, acetylcholine, histamine, serotonin, somatostatin, calcitonin, PACAP, ghrelin, and CCK. This landscape serves as an excellent resource to guide future studies functionally assessing this network.

2. GIPR LOSS OF FUNCTION

2.1. Lessons from knockout mice

2.1.1. Global Gipr knockout mice

The first observation provoking interest in how GIP might regulate body weight in response to overnutrition came from global germ-line

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deletion of *Gipr* in mice ($Gipr^{-/-}$) [3,10]. When fed a standard rodent diet, body weight gain was the same between wild-type (WT) and $Gipr^{-/-}$ mice [3,10]; however, the knockout mice were robustly protected from weight gain when fed a high-fat diet [10]. This protection against high-fat diet-induced obesity in $Gipr^{-/-}$ mice was replicated by multiple labs in subsequent studies [2,50,51]. In addition, crossing the $Gipr^{-/-}$ with leptin-deficient *ob/ob* mice conferred partial protection against weight gain [10]. The decreased weight gain exhibited by $Gipr^{-/-}$ fed a high-fat diet was attributed to decreased fat mass in some [2,10,51] but not all [50] studies. Reduction in lean mass has also been reported to contribute to the decrease in weight gain [51]. How global deletion of the GIPR confers protection against diet-induced obesity remains unclear and debated. Modest decreases in food intake in $Gipr^{-/-}$ mice have been reported; however, these differences disappear when expressed relative to body weight [50], and other studies report no difference in food intake [2]. Energy expenditure measured by indirect calorimetry was slightly elevated in $Gipr^{-/-}$ mice relative to diet-matched control mice [2,10,50]. However, increased energy expenditure of a similar magnitude was also noted in $Gipr^{-/-}$ mice fed standard rodent chow, a condition in which body weight was comparable to diet-matched control mice [2]. Whether this modest increase in energy expenditure contributes to protection against weight gain in high-fat fed $Gipr^{-/-}$ mice but not mice fed a standard diet remains unresolved. It has been reported that $Gipr^{-/-}$ mice use lipids as a preferred energy substrate [10]. However, this observation was transient, being apparent at 3 weeks of high-fat diet but not at 6 weeks, and differences in body weight between WT and $Gipr^{-/-}$ mice were not apparent until after 20 weeks of HFD. Thus, the mechanism by which global deletion of *Gipr* protects against diet-induced obesity has not been convincingly explained. The potential contributions of changes in food intake or energy expenditures will be revisited when examining tissue-specific *Gipr* knockout models.

2.1.2. Adipocyte Gipr knockout mice

A common hypothesis to explain the protective phenotype of $Gipr^{-/-}$ mice is that loss of GIPR activity in white adipose tissue limits lipogenesis. Most support for this hypothesis comes from work in 3T3-L1 adipocytes, where GIP stimulates glucose uptake and lipoprotein lipase activity [10,52,53]. The majority of these experiments utilized supraphysiological concentrations of insulin (often 1 nM) in combination with GIPR agonism (also often used at high concentrations, for example, 100 nM), as GIPR agonism alone fails to enhance lipogenesis. In rat adipocytes, GIP increased free fatty acid re-esterification to produce a net decrease in lipid efflux and attenuated the lipolytic response to isoproterenol [54]. Chronic GIPR agonism led to impaired insulin-stimulated glucose uptake in differentiated human adipocytes [55], which would be expected to impair lipogenesis. However, in contrast to these experiments, GIP was reported to stimulate lipolysis in isolated rodent adipocytes [54,56], differentiated human adipocytes [55], and studies of human subjects [57]. Together, a survey of the literature describing the direct effects of GIP in adipocytes yields confusing and conflicting results, making it difficult to reconcile whether GIPR activity in adipose tissue regulates fat mass.

Information from transgenic mouse models targeting GIPR in adipocytes also failed to shed meaningful insight into the contribution of this adipose tissue toward the high-fat diet-resistant phenotype of $Gipr^{-/-}$ mice. The first transgenic approach employed a strategy of re-expressing *GIPR* selectively in adipose tissue of $Gipr^{-/-}$ mice under control of the *Ap2* promoter [51]. Rescue of the GIPR in adipose tissue failed to normalize body weight gain in response to a high-fat diet, and the modest increase in body weight compared to $Gipr^{-/-}$ was attributed to lean mass, not fat mass. A second study generated an adipose-specific GIPR knockout model through CRE recombinase activity driven by the *Ap2* promoter ($Gipr^{Ap2-/-}$) [40]. The $Gipr^{Ap2-/-}$ line displayed modest reductions in weight gain in response to a high-fat diet, but not to the degree of $Gipr^{-/-}$ mice [10]. Moreover, the reduction in weight in the $Gipr^{Ap2-/-}$ mice was entirely attributed to lean mass, with no differences reported in fat mass. As previously mentioned, the *Ap2* promoter has been documented to have promiscuous expression, with activity in immune cells, endothelial cells, the heart, and brain [41]. Comprehensive documentation of *Gipr/GIPR* levels in either the rescue or knockout model in these tissues was not reported, making it difficult to rule out non-adipocytes from contributing to the lean mass phenotype in these models. However, both models of select modulation of GIPR activity failed to significantly impact adipose tissue mass, strongly hinting that GIP activity in adipocytes, per se, does not regulate lipid or energy metabolism.

The GIPR is also strongly expressed in BAT [47]. Although *Ap2-Cre* has documented activity in brown adipocytes [41], it is interesting to note that the rescue model of *GIPR* failed to increase GIPR expression in BAT [51]. BAT from $Gipr^{-/-}$ mice has increased *Ucp1* levels and significantly less lipid accumulation compared to diet-matched controls [2], and $Gipr^{-/-}$ did not maintain their body temperature during a cold challenge [47], hinting that loss of the GIPR in BAT enhances thermogenesis. To directly test this, BAT-specific *Gipr* knockout mice ($Gipr^{Myf5-/-}$) were generated using *Myf5-Cre* mice [47], which produced Cre recombinase activity in classical brown adipocytes and skeletal muscle; the latter tissue was muted in this experiment since muscle does not express GIPR [47,51,58]. $Gipr^{Myf5-/-}$ mice had similar body weight, fat mass, lean mass, food intake, and energy expenditure relative to control mice when housed at either room temperature or thermoneutrality [47]. A modest decrease in body weight was seen in $Gipr^{Myf5-/-}$ mice fed a HFD that translated into non-significant reductions in lean and fat mass relative to control mice when housed at 4 °C. Differences in energy expenditures were not seen in these groups. The subtly of this effect and the requirement of a strong thermal challenge to produce the phenotype suggest that GIPR activity in the BAT is unlikely to explain the overt phenotype of $Gipr^{-/-}$ mice fed a high-fat diet at room temperature.

2.1.3. Islet GIPR knockout mice

There is evidence to support GIPR activity in islet endocrine cells as an indirect means of regulating adipose tissue mass. In vitro studies describing the adipogenic role of GIP in adipocytes often did so under conditions of supraphysiological insulin concentrations [10,52,53]. However, GIPR agonism in cultured adipocytes without high concentrations of insulin enhanced lipolysis rather than lipogenesis [54–56]. The GIPR is Gαs-coupled with activation increasing cAMP levels, although this mechanism was described in some [36] but not all [53] studies of the adipocyte GIPR. Elevation of cAMP in adipocytes drives lipolysis, a classic mechanism exemplified by the actions pf β-adrenergic receptor agonists such as isoproterenol or epinephrine. However, insulin receptor activation in adipocytes stimulates the activity of phosphodiesterase, which rapidly suppresses cAMP levels and lipolytic rates. Accordingly, the combination of GIP + insulin vs GIP alone has the potential to produce dichotomous signaling events in adipocytes and may explain the failure to reach a consensus on exactly how GIPR agonists influence adipocyte metabolism. In vivo, GIPR agonists potently stimulate insulin secretion through direct actions on β cells. Thus, pharmacologic agents targeting the adipocyte GIPR in vivo will invariably be accompanied by increases in circulating insulin concentrations. The potent lipogenic actions of insulin suggest that

some of the GIPR's potential effects on body weight or composition are governed by β cell activity of GIP.

There is evidence that the insulinotropic actions of GIP in β cells can modulate body weight. β cell *Gipr* knockout mice ($Gipr^{\beta cell-/-}$) showed a reduction in plasma insulin concentrations by ~50% in the postprandial state along with modest reductions in body weight that were attributed to reduced adiposity [30]. To directly test if the reductions in plasma insulin levels limited the gain of adipose tissue mass, control and $Gipr^{\beta cell-/-}$ mice were supplemented with exogenous insulin to normalize the difference in plasma insulin to controls. This intervention ameliorated the differences in fat mass, supporting the argument that the insulinotropic actions of GIP seen in WT but not $Gipr^{\beta cell-/-}$ mice contribute to adipogenesis. However, these experiments were conducted in mice fed a low-fat diet, while $Gipr^{\beta cell-/-}$ mice fed a high-fat diet had similar postprandial insulin concentrations and gained fat mass at the same rate as control mice. Thus, insulin resistance produced by high-fat feeding was sufficient to drive hyperinsulinemia in control and $Gipr^{\beta cell-/-}$ mice and permitted equal weight gain in both groups. Equal weight gain and body composition between high-fat fed control and $Gipr^{\beta cell-/-}$ mice was reported by a separate group [59]. Thus, it is unlikely that eliminating GIPR activity in β cells is responsible for protection against diet-induced obesity presented by $Gipr^{-/-}$ mice.

2.1.4. Immune cell GIPR knockout mice

The GIPR is expressed in a number of hematopoietic-derived bone marrow cells, including T cells, myeloid cells, and myeloid precursors [44]. In loss-of-function GIPR models, including $Gipr^{-/-}$ mice, hematopoietic knockout of *Gipr* ($Gipr^{Tie2-/-}$), or bone marrow transplant (BMT) from $Gipr^{-/-}$ to WT mice, the normal distribution of bone marrow cells was not altered in either chow- or high-fat fed mice [44]. However, loss of bone marrow GIPR expression limited adipose tissue inflammation in response to high-fat feeding [44], suggesting that GIPR signaling in immune cells is proinflammatory in the context of overnutrition. However, reconstitution of hematopoietic cells by BMT from $Gipr^{-/-}$ mice increased weight gain and adiposity in response to a high-fat diet while also enhancing adipose tissue inflammation [46]. These findings contrasted the phenotype of $Gipr^{-/-}$ mice and $Gipr^{Ap2-/-}$ on a HFD and are the only reports to date that loss of GIPR function promotes weight gain. The propensity for diet-induced obesity and increased adipose tissue inflammation was phenocopied in mice with selective deletion of *Gipr* in myeloid cells ($Gipr^{LysM-/-}$) [46]. The results of both models were attributed to decreased energy expenditure arising from a reduction in white adipose tissue beiging and lower rates of thermogenic gene expression; however, thermogenesis or cold tolerance was never directly measured. Moreover, $Gipr^{-/-}$ mice are reported to have superior cold tolerance and enhanced oxygen consumption in response to β3-adrenergic agonists [47], suggesting that global deletion of *Gipr* enhances, not impairs, thermogenesis. Thus, the potential role of GIP in immune cells requires further clarification.

2.1.5. GIP knockout mice

The first studies describing the metabolic effects of reducing GIP levels did so through ablation of intestinal GIP-producing K cells [60]. This was accomplished by generating transgenic mice that used the GIP promoter to express the diphtheria toxin A chain, enabling specific elimination of K cells. Transgenic mice showed specific elimination of intestinal *Gip* and failed to increase insulin levels in response to oral glucose, indicating a significant impairment in the incretin axis. Elimination of K cells did not impact weight gain or food intake when the mice were fed a chow diet. However, transgenic mice had reduced weight gain, less food intake, and increased energy expenditure when fed a high-fat diet. Thus, ablation of K cells produced a similar phenotype compared to $Gipr^{-/-}$ mice.

A more specific approach to target GIP secretion was obtained by replacing the *Gip* gene with *Gfp* to generate GIP-GFP knock-in mice [61,62]. Mice homozygous for the knock-in allele ($GIP^{gfp/gfp}$) had undetectable plasma levels of GIP, whereas heterozygous mice ($GIP^{gfp/+}$) had a ~50% reduction in circulating GIP [61]. Both $GIP^{gfp/gfp}$ and $GIP^{gfp/+}$ mice had impaired oral glucose tolerance due to reduced insulin secretion, further supporting the critical importance of the incretin actions of GIP [61]. No differences in weight were seen in any mice fed a chow diet. The $GIP^{gfp/+}$ mice had reduced weight gain in response to high-fat feeding compared to control mice, which was further magnified in the $GIP^{gfp/gfp}$ mice, suggesting a dose response in weight gain in relation to the attenuation of circulating GIP levels. The reductions in weight were attributed to reduced adiposity and explained by modest increases in energy expenditure and fat oxidation, but not by changes in food intake. Interestingly, crossing the GIP^{gfp} allele onto an *ob/ob* background did not provide any protection against obesity [62], failing to recapitulate the protective effect of crossing $Gipr^{-/-}$ mice on the *ob/ob* background [10]. Together, these studies showed that a reduction in GIP can limit weight gain in response to a high-fat diet similar to global knockout of the *Gipr*, but unlike $Gipr^{-/-}$ mice, loss of GIP cannot protect against obesity induced by impaired leptin signaling.

2.2. Pharmacological antagonism

2.2.1. Peptide antagonists

The remarkable protection against diet-induced obesity originally reported in $Gipr^{-/-}$ mice sparked a number of efforts to antagonize the GIPR pharmacologically. Early efforts utilized modified versions of GIP as competitive antagonist, with (Pro^3)GIP the most common reagent tested [63,64]. Substitution of proline for glutamate residue at position 3 was reported to produce both resistance to DPP4 cleavage and antagonistic properties at the GIPR. Studies in rodents demonstrated that (Pro^3)GIP was able to prevent glucose lowering in response to GIP during a glucose tolerance test, but with modest improvements in glucose tolerance in both WT mice fed a high-fat diet and *ob/ob* mice [65,66]. These results seem at odds, but suggest that the weight reduction potentially leading to improved insulin sensitivity overcame the loss of GIPR signaling in β cells to stimulate insulin secretion. Chronic treatment with (Pro^3)GIP produced reductions in body weight when administered to obese high-fat fed WT mice [66], but not when given to *ob/ob* mice [65], recapitulating a portion of the effects on body weight seen by global deletion of *Gipr* [10]. The mechanism by which (Pro^3)GIP modulates body weight is unclear, but does not appear to be through reductions in food intake. Further complicating the interpretation of (Pro^3)GIP studies are reports that this substitution of the human GIP sequence creates a full agonist at the hGIPR, while rodent sequences are partial agonists at their respective GIPRs [67]. Thus, it appears that (Pro^3)GIP was originally mischaracterized as a full GIPR antagonist and is more accurately a weak partial agonist, refuting the argument that pharmacological antagonism of the GIPR can reduce body weight.

Two naturally occurring GIP peptides have GIPR antagonistic properties when used at supraphysiological concentrations: GIP(3—42) and GIP(3—30) [68]. Of these, the antagonistic actions of GIP(3—30) have been the most thoroughly characterized. Species-specific sequences of GIP(3—30) retain antagonistic properties across rodent and human GIPRs; however, the most potent antagonism is seen when species specificity is matched between peptide sequences and receptors [69].

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In the perfused pancreas, GIP(3—30) partially blocked the increase in insulin, glucagon, and somatostatin induced by GIP(1—42) [70]. It is important to note that the effect of GIP(3—30) to fully block GIPR activity in a physiologically relevant system is incomplete [31], and this peptide is not comparable in potency or reliability to the well-characterized GLP-1R antagonist exendin (9—39) [16]. In rats, administration of GIP(3—30) for 3 weeks did not alter body weight or food intake [71], although the short duration of the study and the delivery of a rapidly cleared peptide complicates the interpretation of these results. However, a different acylated peptide antagonist of the GIPR with a longer half-life that provides chronic antagonism failed to lower body weight over a 10-day period [72].

Studies utilizing GIP(3—30) have been conducted in human subjects to test the role of GIP i) as a glucoincretin [73—75], ii) in adipose tissue metabolism [76], iii) in bone metabolism [77,78], and iv) in the regulation of energy expenditure and feeding behavior during a meal [75]. These studies demonstrated that endogenous GIP signaling significantly contributes to postprandial glucose lowering through elevations in insulin secretion and suggest that GIP is the primary incretin in healthy individuals [75]. Additional work demonstrated that GIP increases adipose tissue blood flow and glucose uptake during a hyperinsulinemic/hyperglycemic clamp, which can be prevented by co-infusion of GIP(3—30) [76]. However, GIP also enhanced insulin secretion under these conditions, supporting a role for insulinotropic actions of GIP to enhance lipogenesis in adipose tissue. Consequently, it is difficult to separate the direct vs indirect roles of GIP in adipose tissue in these studies. However, GIP(3—30) did not impact energy expenditure, substrate utilization measured by indirect calorimetry, or induce any changes in appetite-related measurements during a meal [75], demonstrating that acute GIPR antagonism has a minimal impact on energy balance in healthy individuals. Studies testing the long-term effects of GIP(3—30) have not been conducted.

2.2.2. Antibody antagonists

Two unique antagonizing antibodies of the GIPR have been developed and reported to cause chronic loss of GIPR activity. Gipg013 was the first antibody characterized, and while it was generated to target the hGIPR [79], it is a full antagonist at the mGIPR [26,80]. Intracerebroventricular (icv) injection of Gipg013 every other day in obese WT mice led to dramatic decreases in body weight over a two-week period driven by decreases in food intake [80]. Weight loss was not observed when administering Gipg013 to obese *ob/ob* mice, leading the authors to assess the relationship between GIP and leptin in the brain. Icv administration of GIP at supraphysiological concentrations (50—5000 nM) prevented leptin signaling in brain slices ex vivo [80], while icv administration of GIP in vivo caused proinflammatory signaling that inhibited insulin and leptin signaling in the hypothalamus [81]. Peripheral injection of Gipg013 at 15 mg/kg did not impact body weight in these studies [80]. Separate studies demonstrated that peripheral administration of Gipg013 at a higher dose (30 mg/kg) prevented weight gain when commenced with the onset of high-fat feeding in mice and had modest effects to induce weight loss in already obese mice [26]. The changes in body weight were attributed to decreases in fat mass, not lean mass, and were caused by decreases in food intake, not energy expenditure. The effect of Gipg013 on body weight was modest (~20% decreases) compared with GLP-1R agonism with dulaglutide in a parallel group of mice (50—90% decreases). These results were largely in line with the effects of an another GIPR-antagonizing antibody that was developed to target the mGIPR (muGIPR-Ab) [59,82,83]. Peripheral treatment of high-fat fed obese mice with muGIPR-Ab produced modest (~5%) decreases in body weight, while dulaglutide given over the same time period induced ~15% weight loss. The effects of muGIPR-Ab on body weight were more pronounced with treatment initiated simultaneously with high-fat feeding and attributed to decreases in food intake rather than energy expenditure. Overall, the metabolic effects of these two antibodies were remarkably similar [26], with both demonstrating that chronic GIPR antagonism in mice can induce modest decreases in body weight.

A striking observation in studies using muGIPR-Ab came from the combination of GIPR antagonism with GLP-1R agonism [59]. This combination produced a synergistic effect on body weight, nearly doubling the amount of weight loss induced by GLP-1R monoagonism alone. This relationship was independent of the GLP-1R agonist used, as synergy was demonstrated with exendin-4, dulaglutide, and liraglutide. A similar relationship was reported with Gip013, albeit without as pronounced an effect as with muGIPR-Ab [26]. The combination of Gipg013 and dulaglutide had a subtle effect to limit weight gain compared to dulaglutide alone (58% vs 49%, respectively), but this was not statistically significant. These differences between studies may be due to the species differences in the antagonists used. muGIPR-Ab was developed to target the mGIPR and is a more efficacious antagonist of the GIPR in mice, while Gipg013 was developed to target the hGIPR. Indeed, a subtle decrease in the antagonistic actions of Gipg013 was noted at the end of a 20-week study [26], highlighting that muGIPR-Ab may have achieved a higher level of antagonism than Gipg013 in mice, leading to more demonstrable synergy when combined with a GLP-1R agonist. Furthermore, treatment of obese non-human primates with hGIPR-Ab produced additive effects on weight loss when combined with dulaglutide but did not approach the synergy seen with combination treatment in mice [59]. Nonetheless, taken together, it is clear that chronic antagonism of the GIPR with various antibodies produces some degree of weight loss in obese models. Neither antagonizing antibody produced hyperglycemia in mice, but rather led to modest reductions in blood glucose [26,59,82]. Potential explanations for the modest improvement in glucose tolerance include a compensatory increase in GLP-1R signaling [26], modest reductions in body weight, and a positive improvement in the adipokine profile, enabling enhanced insulin sensitivity [82].

2.3. Summary of GIPR loss-of-function studies

Nearly all of the evidence originating from studies that utilized various strategies to reduce or eliminate GIPR signaling reported protection against diet-induced obesity (Table 1). Most studies showed no differences in body weight from blocking GIPR signaling when mice were fed a standard rodent chow, highlighting the necessity for overnutrition to reveal a phenotype. The mechanisms facilitating the decrease in weight gain when GIPR signaling is blocked remain unclear. Much of the data from genetic loss of *Gipr* hints at elevations in energy expenditure potentially due to enhanced thermogenesis or ambulatory activity [2,47], while food intake was often reported as similar to controls. However, pharmacological antagonism of GIPR presents subtle decreases in food intake, without appreciable differences in energy expenditure [26,59]. Thus, while the overall effects on body weight are consistent among models, the mechanisms to explain this are not. Adding to this complexity are reports that loss of GIPR most often results in decreased adipose tissue mass [2,10,26,59], but

Table 1 — Summary of the effects of different interventions that target the GIP/GIPR on body weight and compositions. HFD: high-fat diet; RT: room temperature; BMT: bone marrow transplant; IP: intraperitoneal; ICV: intracerebroventricular.

		Model		Effect on BW	Proposed Mechanism	Ref
Loss of Function	Genetic Model	*Gipr* KO	Germ line, global deletion of *Gipr*	- No impact on chow diet - Reduced gain on HFD due to fat mass - Reduced gain on *ob/ob* background due to fat mass	- Increased energy expenditure - Reduced lipid uptake into adipose tissue	[10]
		Gipr:Glp1r KO	Germ line, global deletion of *Gipr* and *Glp1r*	- No impact on chow diet - Reduced gain on HFD due to fat mass	- Increased energy expenditure	[2]
		Gip KO	Germ line, global deletion of *Gip*	- No impact on chow diet - Reduced gain on HFD due to fat mass - No impact on *ob/ob* background	- Increased energy expenditure	[61,62]
		K cell KO	Diphtheria toxin driven by *Gip*	- No impact on chow diet - Reduced gain on HFD due to fat mass	- Increased energy expenditure	[60]
		β cell *Gipr* KO	*Mip-CreERT* x *Gipr* flox	- Reduced gain on chow diet due to fat mass - No impact on HFD	- Reduced insulin secretion	[30,59]
		Adipocyte *Gipr* KO	*Ap2-Cre* x *Gipr* flox	- No impact on chow diet - Reduced gain on HFD due to lean mass (no changes in fat mass)	- Increase adipose tissue inflammatory signaling	[40]
		Adipocyte *Gipr* KO	*Adipoq-Cre* x *Gipr* flox	- No effect on body weight, but small reductions in fat mass	- Reduced lipid uptake into adipose tissue	[82]
		Brown Adipose *Gipr* KO	*Myf5-Cre* x *Gipr* flox	- No impact when housed at RT - Decreased gain at 4 °C	- Unclear	[47]
		Bone marrow *Gipr* KO	BMT from *Gipr* KO to WT mice	- Increased weight gain on HFD due to fat mass	- Decreased energy expenditure - Increased adipose tissue inflammatory signaling	[46]
		Immune cell *Gipr* KO	*LysM-Cre* x *Gipr* flox	- Increased weight gain on HFD due to fat mass	- Decreased energy expenditure - Increased adipose tissue inflammatory signaling	[46]
	Pharmacology	(Pro_3)GIP	Peptide antagonist against GIPR	- Reduces gain on HFD due to fat mass - Decreased weight in obese mice and *ob/ob* mice due to fat mass	- Unclear	[65,66]
		GIP(3—30)	Peptide antagonist against GIPR	- No impact		[71]
		Acyl GIPR antagonist	Peptide antagonist against GIPR	- No impact		[72]
		Gipg013	Antibody antagonist against GIPR (IP)	- Reduced gain on HFD due to fat mass - No impact on obese mice	- Reduced food intake - Increase energy expenditure	[26]
		Gipg013	Antibody antagonist against GIPR (ICV)	- Reduced weight in obese mice due to fat and lean mass	- Reduced food intake	[80]
		Mu-GIPR-Ab	Antibody antagonist against GIPR	- Reduced gain on HFD due to fat mass - Decreased weight in obese mice due to fat mass	- Reduced food intake	[59]
Gain of Function	Genetic Model	GIP overexpression	*Gip* driven by metallothionein promoter	- Reduced gain on HFD due to fat mass	- Reduced food intake - Increased energy expenditure	[87]
		Adipose *Gipr* rescue	*Ap2-Gipr* x *Gipr* KO	- Increased weight due to lean mass	- Unclear	[51]
		β cell *Gipr* rescue	*RIP-Gipr* x *Gipr* KO	- No impact		[51]
	Pharmacology	$(D\text{-}ala_2)$GIP	GIPR agonist	- No impact		[91]
		(Aib_2)GIP	GIPR agonist	- Decreased weight in obese mice	- Reduced food intake	[72]
		ZP4165	GIPR agonist	- No impact on *db/db* mice		[94]
		LA agonist	GIPR agonist	- No impact		[108]
		LA-GIPRA	GIPR agonist	- No impact		[95]

models that target adipose tissue GIPR alter lean mass, not fat mass [40,51]. Nearly two decades of research following the original observation that loss of GIPR limits obesity in mice [10] have failed to produce a unifying explanation for these results. It stands to reason that the original hypothesis that GIP enhances adipose tissue lipogenesis to drive obesity derived from the $Gipr^{-/-}$ phenotype [10] does not sufficiently explain what is clearly a more complex biology.

3. GIPR GAIN OF FUNCTION

Currently available evidence suggesting that elevated GIP levels drive increased body weight and adiposity is only associative. The stimulation of GIP secretion by fat-containing meals led to speculation that GIP must be linked to fat deposition and obesity. For instance, lipids potently stimulate GIP secretion in humans [84], and high-fat feeding

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increases the expression of *Gip* in the intestine, which stimulates K cell hyperplasia and raises the circulating levels of GIP in rodents [84,85]. In humans, the obesity level correlates with circulating concentrations of GIP [86]. As previously outlined, the combination of GIPR agonism in adipocytes along with the insulinotropic actions of β cells produces a lipogenic environment in adipose tissue. However, the evidence to support a direct obesogenic role of GIP is largely derived from the previously discussed *Gipr*$^{-/-}$ mouse model. In this section, studies examining the effects of GIPR agonism are reviewed to contextualize the potential role of GIPR gain of function for controlling body weight.

3.1. Transgenic overexpression of GIP

Mice with conditional expression of *Gip* under control of metallothionein promoter had ∼1000-fold increases in circulating GIP levels [87]. When fed a standard rodent diet, transgenics did not differ in body weight from controls. However, provision of a high-fat diet revealed that GIP overexpression limited weight gain, resulting in reduced adiposity and improved glucose tolerance. The protection against diet-induced obesity in the transgenic mice was attributed to reductions in food intake and their energy expenditure was similar to controls. This study demonstrated that obtaining pharmacological levels of circulating GIP protects against weight gain and obesity, ultimately improving glucose homeostasis relative to obese control mice.

3.2. Pharmacological agonism

3.2.1. Acute GIPR agonism

While the effects of acute GIPR agonism do not speak directly to changes in body weight or composition that could potentially occur with chronic increases in GIPR activity, these studies can provide insight into plausible mechanisms by which GIP could regulate the systems that determine energy balance. For instance, infusion of GIP during a meal to produce ∼2x increase in normal prandial GIP levels did not alter energy intake or energy expenditure in healthy humans [88]. However, when the same concentrations of plasma GIP were reached by co-infusion with intralipids, concentrations of FFAs were reduced compared to controls infused with saline and intralipids, suggesting that acute increases in GIP either enhance fatty acid uptake or limit lipolysis in adipose tissue [89]. Additional studies have shown that elevating GIP levels during a hyperinsulinemic/hyperglycemic clamp increases adipose tissue blood flow, glucose uptake, and FFA re-esterification to ultimately increase lipid storage in adipose tissue [88]. Interestingly, GIP infusion alone in the absence of hyperinsulinemia/hyperglycemia failed to alter any of these parameters, highlighting the important interactions between GIP and insulin for adipose tissue anabolism [88]. However, other studies comparing the acute effects of GIP in healthy vs T2D patients reported that the direct effects of GIP on adipose tissue metabolism are only seen when insulin levels are low [90]. The conflicting reports of studies with acute administration of GIP in humans are discrepant and preclude firm conclusions.

3.2.2. Chronic GIPR agonism

Multiple studies have investigated the role of chronic GIPR agonism on body weight and glucose tolerance in preclinical models. Chronic agonism achieved by (D-Ala$_2$)GIP, a long-acting DPP4 resistance GIP analog given at 24 nmol/kg twice daily for 8 weeks, did not alter body weight, food intake, body composition, or energy expenditure in high-fat fed mice [91]. Chronic agonism achieved with daily administration of (Aib$_2$)GIP, a peptide similar to (D-Ala$_2$)GIP, given at 2 nmol/kg for 14 days also did not alter body weight or food intake in obese mice [92]. Modest decreases in body weight and food intake were seen when an acylated GIP analog was given to obese mice at a daily dose of 10 nmol/kg [93], suggesting a greater effect on metabolism is achieved by either the higher dose or greater exposure enabled by the acylated peptide. In support of this, daily administration of various acylated GIP analogs at doses ranging from 20 to 100 nmol/kg produced significant decreases in body weight and food intake in obese control mice, but not *Gipr*$^{-/-}$ mice [72]. However, a different acylated GIP analog (ZP4165, 10—50 nmol/kg) was weight neutral after 4 weeks of treatment in obese *db/db* mice, although the higher dose (50 nmol/kg) was additive with liraglutide in preventing weight gain [94]. Thus, chronic agonism of the GIPR in mice is either weight neutral or weight lowering depending on the level of agonism achieved. There is no evidence to support chronic agonism of the GIPR inducing weight gain or adiposity in preclinical models.

Additive or synergistic effects on reducing body weight in mice are achieved when GIPR agonists are co-administered with GLP-1R agonists [72,92,94], although the mechanism explaining this effect remains elusive. Nonetheless, the consistent observation that combining GIPR and GLP-1R agonism greatly reduces weight loss has spurred the investigation of single peptide agonists that have dual activity at both receptors for treating obesity and diabetes [7]. The most advanced of these molecules is tirzepatide, a GIPR/GLP-1R co-agonist currently in phase 3 clinical trials that has been shown to induce substantial weight loss in both preclinical [95] and clinical studies [15]. Interestingly, investigation into tirzepatide's pharmacology has revealed imbalanced engagement between the incretin receptors, with a greater degree of engagement of the GIPR compared to the GLP-1R, and a pharmacological profile that more closely resembles GIP at the GIPR than GLP-1 at the GLP-1R [96]. Together, the data from monoagonism of the GIPR along with evidence from multireceptor agonists that incorporate GIPR activity indicate that chronic GIP activity does not promote weight gain or obesity, but rather has the potential to be weight lowering.

The efficacy of multireceptor agonists for the GIPR and GLP-1R to reduce both glycemia and weight gain raises the question of whether and how targeting more than one receptor produces synergy. Both GIPR agonism [92] and antagonism [26,59] is more efficacious when combined with agonists for the GLP-1R. β cells express both receptors, yet the combination of GIP and GLP-1 does not produce synergistic effects on insulin secretion. Instead, the combination is additive at best when peptides are given at physiological levels [97] or that GIP fails to enhance GLP-1 stimulated insulin secretion when peptides are administered at pharmacological levels [98—100]. Since the GIPR and GLP-1R are class B GPCRs that signal predominantly through cAMP, maximally stimulating the GLP-1R with pharmacological doses may saturate this signaling pathway, leaving very little room for additional signaling through the GIPR.

Another location with potential co-expression of GIPR and GLP-1R is the brain. Evidence for this was generated by RNAscope analysis in mouse and human samples, demonstrating that at least a subpopulation of hypothalamic nuclei are *Gipr:Glp1r*+ [29]. Potential overlap of incretin receptor expression in other brain regions was not reported and remains unknown. Functionally, simultaneous activation of both neuron populations achieved through designer receptor exclusively activated by designer drugs (DREADD) expression in these cells failed to produce additivity compared to monoagonism alone [29]. This suggests that incretin receptor activation in the hypothalamus, a key central node controlling food intake, is not additive. These findings in mice agree with studies in humans that reported that the combination of GIP and GLP-1 infusion did not decrease food intake, alter appetite, or enhance energy expenditure beyond GLP-1 infusion alone [98,99].

There are very few remaining tissues that potentially express both incretin receptors. Potential locations include cardiomyocytes, endothelial cells, enterocytes, and immune cells. However, the expression of either the GLP-1R or GIPR in these cell populations is scattered and often difficult to pinpoint, decreasing the likelihood of co-expression of both receptors.

An alternative hypothesis to synergy within the same cell as a way to explain the effects of MRA is that modulation of GIPR activity enables targeting of key metabolic tissues that do not express the GLP-1R. Candidate cell types include both white and brown adipocytes, α cells, and adrenal glands. Furthermore, a detailed profile of GIPR expression in the brain may reveal unique neuron populations that are GLP-1R negative, indicating a greater coverage of neural networks through combined incretin action. Understanding the contributions of GIPR activity in these GLP-1R negative tissues that are intimately linked with glucose homeostasis and the regulation of body weight may be key to answering how the combination of targeting the GIPR along with GLP-1R agonists is so efficacious.

3.3. Summary of GIPR gain of function studies

The majority of studies that enhanced GIPR signaling reported limited effects on body weight (Table 1). Most studies showed that GIPR pharmacology was weight neutral in obese models, while no studies have reported that GIP enhances weight gain. Achieving a high level of GIPR agonism over a sustained period promotes weight loss, although not to the level achieved by GLP-1R agonism. The combination of GIPR and GLP-1R agonism synergistically fosters weight loss, highlighted by the clinical outcomes achieved by tirzepatide [15]. The mechanism by which combined incretin action mediates weight loss remains unknown. However, the sum total of studies reporting on GIPR agonism did not support the long-standing view that GIP is an obesogenic hormone that drives weight gain and obesity, a view that originally stemmed from the phenotype of *Gipr*$^{-/-}$ mice. Indeed, the most consistent observation is that *both* GIPR agonism and antagonism engage in mechanisms that promote negative energy balance and ultimately reduce body weight.

4. HYPOTHESES TO RECONCILE AGONISM VS ANTAGONISM

Current models of a biological role of GIP in regulating body weight are not simple or clear. The experimental evidence in this area is discordant and conclusions are often contingent on the study design or reagents. This leaves the issue of how to apply GIPR signaling to pharmacology and whether to pursue agonism or antagonism of the GIPR as open questions. As previously outlined, there is evidence to support both approaches as having potentially meaningful effects on weight and metabolic health. But how can both agonism and antagonism of the same system produce a similar outcome? This is a scientifically interesting question, but the apparent paradox has become a discouraging "dead end," limiting progress in what could be a fruitful area of translational research. Often overlooked is that a similar paradox is present in gain- and loss-of-function studies targeting the GLP-1R. The clinical success of GLP-1R agonists to cause weight loss support enhancing GLP-1R signaling to treat obesity [6]. However, both *Glp1r*$^{-/-}$ mice [2,101] and chronic antagonism of the GLP-1R [26] are protective against diet-induced obesity. Thus, the agonism-antagonism paradox actually holds for both incretins. In this report, two leading hypotheses are presented that could resolve the question of how best to modulate incretin signaling to reduce body weight.

4.1. The apparent incretin compensation effect

The development of incretin receptor knockout models revealed what appeared to be overlap in these two signaling axes as highlighted by an increase in the effects of one incretin following genetic elimination of the receptor for the other. This was first noted in a study showing markedly enhanced insulin secretion in response to administration of GLP-1 to *Gipr*$^{-/-}$ mice [102]. A further study showed that this relationship is bidirectional, as *Glp1r*$^{-/-}$ mice had enhanced glucose lowering and insulin secretion when stimulated with GIP [103]. This effect seems to be medicated at the level of β cells since islets from *Gipr*$^{\beta cell-/-}$ mice have a similar augmented response to GLP-1 [30] as do *Gipr*$^{-/-}$ islets. Consistent with a cell autonomous response, *Gipr*$^{-/-}$ and *Gipr*$^{\beta cell-/-}$ mice have similar fasted and stimulated GLP-1 levels compared to control mice, ruling out increased L cell secretion as an explanation of the greater responsiveness to GLP-1. Moreover, the expression of *Glp1r* in islets is unchanged in both knockout models, suggesting that enhanced sensitivity to GLP-1 is not due to greater numbers of GLP-1R on the surface of β cells that do not make GIPR. The increased responsiveness to one incretin in the absence of signaling by the other may represent compensation, that is, a means of maintaining the incretin effect, or it may due to intracellular signaling responses that are not linked to any physiologic or homeostatic adaptation. At present, there is strong evidence for a system of mutual compensation between the incretins, but the mechanism remains unclear.

The relationship between incretin receptors has been extended to weight loss, although only partially recapitulating the response of β cells. To wit, GLP-1R agonists produced substantially more weight loss when administered to *Gipr*$^{-/-}$ mice than wild-type mice. However, a similar augmentation of weight loss was not seen in *Glp1r*$^{-/-}$ mice given GIP [72]. The enhanced weight loss in response to GLP-1R agonism in *Gipr*$^{-/-}$ was due GIPR outside β cells since it was not apparent in *Gipr*$^{\beta cell-/-}$mice given dulaglutide [59]. The likely site of mediation of this effect is the nervous system, and it is plausible that eliminating the GIPR in the brain enhances GLP-1R activity to alter body weight. In the absence of pharmacologic GLP-1R agonists, *Gipr*$^{-/-}$ mice have normal food intake, indicating that endogenous GLP-1R signaling is not sufficient to change energy balance or is compensated for by other mechanisms. It is possible that enhancing CNS GLP-1R signaling drives sympathetic output to increase thermogenesis in *Gipr*$^{-/-}$ mice, and in fact, central GLP-1R signaling has been proposed to regulate thermogenesis in BAT [104,105]. Regardless, preclinical studies provide support for interactions of incretin receptor signaling, probably in the brain, to regulate body weight.

Enhanced sensitivity to GLP-1R agonists was also seen following chronic pharmacological antagonism of the GIPR. This was first documented by the synergistic effect on weight loss in DIO mice produced by the combination of muGIPR-Ab and liraglutide [59]. The synergism on weight loss was not driven by reduced food intake, and energy expenditure was not measured in mice receiving the combined treatment; the mechanism for enhanced weight loss in this experiment was unclear. Separate studies also reported that GIPR antagonism obtained with Gipg013 given either icv [80] or peripherally [26] did not enhance GLP-1R mediated reductions in food intake. Interestingly, chronic pharmacological antagonism of the GLP-1R with a blocking antibody (Glp1r0017) enhanced the ability of a GIPR agonist to reduce food intake [26]. Thus, pharmacological antagonism of incretin receptors yields similar plasticity in the incretin axis originally documented in studies of incretin receptor knockout mice. This emphasizes that the compensatory increase in signaling between incretin receptors

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is due to loss of function per se, rather than non-biological, collateral abnormalities resulting in genetic modifications. But does plasticity in the incretin system account for the protection against diet-induced obesity seen with either genetic knockouts of a single incretin receptor [2,10] or chronic antagonism of a single incretin receptor [26]? Double incretin receptor knockout (DIRKO) mice gained significantly less weight on a high-fat diet [2], and chronically antagonizing both incretin receptors prevented weight gain to a greater extent compared to a single antagonism [26]. Thus, compensation between incretin receptors cannot fully explain the protective phenotype that arises with loss of activity of a single incretin receptor, suggesting that enhanced GLP-1R activity is not solely responsible for the phenotype of *Gipr*$^{-/-}$ mice or the metabolic effects of GIPR antagonist.

While more research is needed to provide clarity in this area, loss-of-function studies of incretin receptors provide evidence of metabolic plasticity and compensation. In other words, eliminating a signaling axis involved in the metabolic response to food intake seems to enhance other systems in the energy balance network. Moreover, since energy balance is a tightly regulated parameter, it seems likely that this response is an attempt to maintain homeostasis. It is also evident from decades of research that the incretin system is primary in the control of postprandial homeostasis. Yet, complete loss of incretin signaling [2,26] lends greater protection against weight gain and improves glucose tolerance when challenged with high-fat feeding. Since the incretin system integrates with additional factors that regulate metabolic homeostasis, it is also plausible that removing incretin signaling places additional stress on these complementary systems. This creates a level of metabolic inefficiency as the burden of postprandial metabolism is carried by regulatory pathways that did not evolve to be the primary factors maintaining this system (Figure 1). However, without clarifying these potential compensatory pathways, it is difficult to predict how these inefficiencies in metabolism manifest. This inefficiency could explain the subtle increase in energy expenditure common to loss of incretin receptor signaling and the resulting protection against diet-induced obesity. Some potential candidate mechanisms to explain an increase in energy expenditure could include altering substrate utilization, changing body temperature, or futile cycles. However, while this remains an untested hypothesis, the potential to explain elements of body weight regulation provide impetus to investigate mechanisms that facilitate plasticity within the incretin system.

4.2. Chronic agonism may equal antagonism

A second hypothesis that has been advanced to explain the role of GIPR signaling on body weight is that chronic agonism of the GIPR produces desensitization of the GIP system and ultimately the same result as a GIPR antagonist [82] (Figure 2). GPCR desensitization, in which an initial stimulus with a ligand reduces the response to a subsequent stimulus, has been shown to occur for the GIPR in 3T3-L1 adipocytes [106]. This study demonstrated that an initial GIP stimulus led to internalization of the GIPR, with slower recycling rates back to the plasma membrane to ultimately reduce the amount of GIPR available for subsequent ligand interactions. Of note, the recycling rate was even slower when the cells were transfected with the E354Q GIPR variant. The slower recycling rate of the E354Q variant would be expected to enhance agonist-induced desensitization and produce an effect closer to a GIPR knockout. In line this premise, the E354Q variant was associated with a lower BMI in GWAS studies [107]. One limiting characteristic of this work was the very high concentrations of GIP (100 nM) used to induce desensitization, which were likely several orders of magnitude greater than concentrations achieved even by GIP pharmacology. A similar desensitization was recently reported in response to long-acting GIPR agonists in the adipose tissue of mice. Differentiated mouse or human preadipocytes exposed to 1 μM (D-Ala^2)-GIP for 24 h in culture had a decreased cAMP response to subsequent GIPR agonism, indicating desensitization of the GIPR

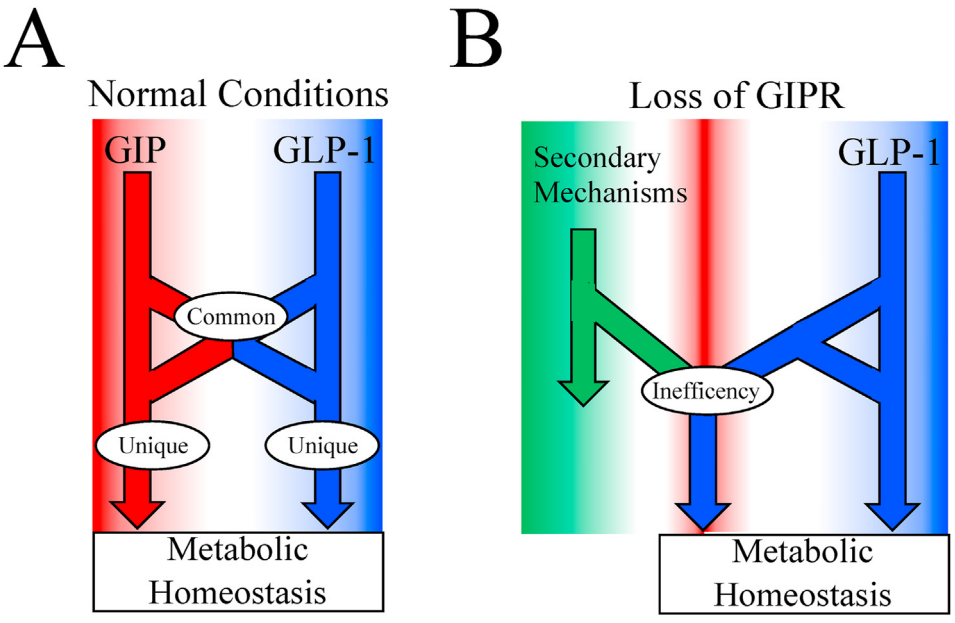

Figure 1: Metabolic Inefficiency Resulting from Compensation: A Hypothesis. A) The incretin system contains both overlapping and unique functions in metabolic control. B) Loss of a single incretin axis forcing compensatory actions in the complementary axis to maintain homeostasis. Furthermore, loss of both incretin signaling pathways places metabolic burden on systems that did not evolve to be primary in maintaining homeostasis. Reliance on "secondary mechanisms" creates a level of metabolic inefficiency that drives a decrease in body weight.

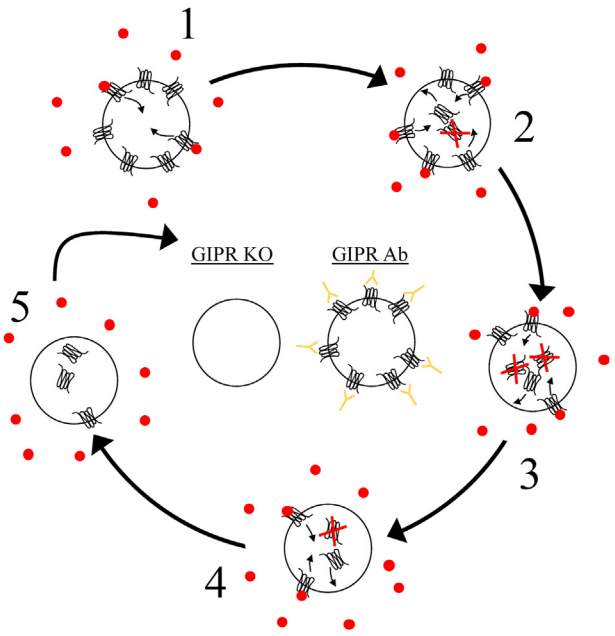

Figure 2: Chronic Agonism Equals Antagonism: A Hypothesis. 1) Agonism of the GIPR leads to internalization of the number of receptors that is proportional to the concentrations of agonists. 2) Some receptors will recycle back to the membrane, while others will degrade. 3–5) Chronic agonism eventually decreases the number of receptors present on the membrane to effectively resemble loss of function caused by either *Gipr* knockout or GIPR antagonism (GIPR Ab).

[108]. Mechanistically, the authors showed enhanced internalization of the GIPR in response to chronic agonism in transfected HEK293T and CHOK1 cells through ligand-binding assays, demonstrating that chronic GIPR agonisms reduced the level of available receptors (Figure 2). Overall, the interpretation of these data is hampered by a number of limitations. First, the use of extremely high peptide concentrations (10–1000 nM) questions the applicability to GIP physiology or even pharmacology. Indeed, it was recently estimated that current GIPR pharmacology achieved by the highest clinical doses of tirzepatide (15 mg) produced free peptide concentrations of ~0.7 nM [96], several orders of magnitude below those used to demonstrate receptor desensitization [82,106]. Directly measuring the GIPR numbers in primary cells following chronic agonism has not been conducted in part due to the lack of quality reagents required to do so. Still, if agonist-induced desensitization ultimately creates a state of GIPR signaling analogous to *Gipr* knockout or GIPR antagonists, it would explain how both chronic agonism and antagonism of the receptor produce weight loss.

An argument against this hypothesis is based on recent evidence that continuous exposure to high concentrations of GIP fails to produce tachyphylaxis in either healthy subjects or people with T2D [109]. This is similar to the internalization properties of the GLP-1R, which undergoes similar or even more efficient internalization compared to the GIPR [96] without evidence of tachyphylaxis. The maximum duration of GIP infusion was 3 h and the GIP concentrations were supraphysiological, but potentially not as high as pharmacological dosing of GIPR agonists in preclinical studies. Furthermore, the primary outcome measurement was insulin secretion, rather than an outcome measuring GIPR activity in adipose tissue or energy balance. Thus, there is clearly much more work to be done to identify if agonist-induced desensitization occurs with respect to the GIPR in humans in vivo and the conditions that potentially drive this.

5. CONCLUSIONS AND FUTURE DIRECTIONS

The contribution of GIP to metabolic homeostasis was first identified by John Brown et al. in the 1970s [110,111], yet nearly 50 years, later it seems there is more confusion than clarity on exactly how GIP controls metabolism. The effects of GIPR signaling on energy balance and body weight exemplifies several fundamental debates about GIP's role in physiology and pharmacology. Similar to most scientific debates, there is substantial evidence on both sides of the GIPR and body weight issue, and cogent arguments can be made that both gain and loss of function of GIP can provide positive outcomes on body weight and glucose control. To reconcile this paradox, it is essential to first fully understand GIP's complete metabolic actions. Key outstanding questions remain with respect to the extent and specifics of GIP's direct action on adipocytes, and the current body of evidence stems primarily from artificial models, heavily outweighing and often in conflict with the data from primary tissue systems. Other frontiers that have significant potential to produce meaningful advances in this area are understanding GIP's potential role in the brain and immune system, with emerging evidence suggesting the meaningful impact of the GIPR. The interaction between the GIPR and GLP-1R is clearly important, based on the remarkable efficacy of therapeutic modalities that combine GLP-1R agonism with GIPR agonism. This line of research suggests that the incretin system does not merely consist of two redundant peptides, but rather achieves full effects when both peptides are fully engaged. However, how and why this occurs remains a mystery that needs to be solved to truly substantiate this statement. Furthermore, this does little to reconcile the equally efficacious outcomes that result from GLP-1R agonism combined with GIPR antagonism. Thus, the field currently remains stuck at a stage of phenomenology whereby modulating GIPR activity in either direction can in fact impact body weight. Without a deeper understanding of GIP's biology, the debate over agonism vs antagonism cannot be truly resolved, nor can the potential consequences of either intervention be predicted. Hopefully it does not take another 50 years to achieve the necessary insight into GIP's metabolic role to guide its correct therapeutic use for treating metabolic disease.

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CONFLICT OF INTEREST

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