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GLP-1 receptor agonism and GIP receptor antagonism induce substantial alterations in enteroendocrine and islet cell populations in obese high fat fed mice

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ABSTRACT

Effects of sustained activation of glucagon-like peptide-1 (GLP-1) receptors (GLP-1R) as well as antagonism of receptors for glucose-dependent insulinotropic peptide (GIP) on intestinal morphology and related gut hormone populations have not been fully investigated. The present study assesses the impact of 21-days twice daily treatment with the GLP-1R agonist exendin-4 (Ex-4), or the GIP receptor (GIPR) antagonist mGIP(3−30), on these features in obese mice fed a high fat diet (HFD). HFD mice presented with reduced crypt depth when compared to normal diet (ND) controls, which was reversed by Ex-4 treatment. Both regimens lead to an enlargement of villi length in HFD mice. HFD mice had increased numbers of GIP and PYY positive ileal cells, with both treatment interventions reversing the effect on PYY positive cells, but only Ex-4 restoring GIP ileal cell populations to ND levels. Ex-4 and mGIP (3−30) marginally decreased GLP-1 villi immunoreactivity and countered the reduction of ileal GLP-1 content caused by HFD. As expected, HFD mice presented with elevated pancreatic islet area. Interestingly, mGIP(3−30), but not Ex-4, enhanced islet and beta-cell areas in HFD mice despite lack of effect of beta-cell turnover, whilst Ex-4 increased delta-cell area. Co-localisation of islet PYY or GLP-1 with glucagon was increased by Ex-4, whilst islet PYY co-immunoreactivity with somatostatin was enhanced by mGIP(3−30) treatment. These observations highlight potential new mechanisms linked to the metabolic benefits of GLP-1R agonism and GIPR antagonism in obesity.

1. Introduction

Dysregulation of the incretin hormone system in obesity and related diabetes has profound effects on metabolic function. Specifically, reduced glucagon-like peptide-1 (GLP-1) secretion and impaired GLP-1 and, especially glucose-dependent insulinotropic peptide (GIP) receptor signalling leads to diminished insulin secretion, impaired glucose uptake and increased hepatic glucose production [1]. The blunted GLP-1 response also contributes to decreased satiety and increased appetite, further exacerbating weight gain and obesity [2]. It follows that long-acting drugs mimicking the action of GLP-1 are now clinically approved for both obesity and type 2 diabetes [3].

In terms of clinically exploitable effects of GIP in obesity and diabetes, the picture is less clear. Thus, activation and inhibition of GIP receptor (GIPR) signalling impart significant metabolic benefits, both alone [4,5] and in combination with GLP-1 receptor (GLP-1R) activation [6,7]. Notably however, there is now a suggestion that chronic GIPR agonism leads to receptor desensitisation, thereby mimicking the observed benefits of GIPR antagonism [8,9]. This is in keeping with the early idea that GIP plays a role in obesity [10] and with observations that GIPR knockout (KO) mice fed a high fat diet are resistant to obesity [11]. Further to this, there are important species-specific divergences within the GIP system that need to be considered, with the human GIP sequence acting only as a comparatively weak partial agonist in rodent systems [12]. Consequently, mouse GIP(3−30) (mGIP(3−30)), but not human GIP(3−30) (hGIP(3−30)), is demonstrated to be a highly effective molecule to inhibit GIPR activity in mice [13], with hGIP(3−30) more applicable in the human setting [14].

Therefore, in the current study we have employed sustained injection of either exendin-4 (Ex-4) to activate GLP-1Rs, or mGIP(3−30) to antagonise GIPRs, in our model system. Although both approaches are known to bring about well-known improvements in metabolism, enteroendocrine cellular adaptations in response to these interventions has not been examined in detail. In this regard, there is clear evidence

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for both pancreatic islet and enteroendocrine cell (EEC) adaptations in obesity and diabetes, that may be linked to disease onset and progression [15]. As such, changes in both islet beta-cell and EEC numbers as well as secretory function have been documented [16]. Thus, the primary objectives of the current study were to investigate the impact of (Ex-4) and mGIP(3−30) therapy on intestinal morphology and hormone content as well as gut cell hormone immunoreactivity profiles in mice fed a high fat diet (HFD). Secondary objectives included validation of benefits of the treatment interventions on metabolic control as well as assessment of pancreatic islet architecture. Together these studies will help uncover the influence of changes in intestinal morphometry in relation to the positive effects of GLP-1R agonism and GIPR antagonism under situations of high fat feeding.

2. Materials and methods

2.1. Peptides

Ex-4 and mGIP(3−30) were purchased from SynPeptide (Shanghai, China) at greater than 95% purity. In-house confirmation of peptide purity and molecular weight was carried out by reversed-phase high-performance liquid chromatography (RP-HPLC) and matrix-assisted laser desorption/ionisation–time-of-flight mass spectrometry (MALDI-ToF MS), as previously described [13].

2.2. Animals

Female Swiss NIH mice (4–6 weeks old, Envigo, UK) were housed individually in air-conditioned room at 22 ± 2 °C with 12 h light and dark cycle and ad libitum access to standard rodent diet (10% fat, 30% protein and 60% carbohydrate; Trouw Nutrition, Northwich, UK) and drinking water. NIH mice are an outbred strain of mice and therefore more closely resemble the genetic differences present within the human population, with use of female mice confirming lack of sex-specific dimorphic effects of Ex-4 and mGIP(3−30), since previous studies with these peptides have largely employed male mice [13,17]. At 9 weeks of age, mice were fed a HFD (45% fat, 35% carbohydrate and 20% protein; 26.15 kJ/g, Special Diet Services, UK) for 14 weeks, with a separate group maintained in normal diet (ND) throughout and serving as gold standard controls. Following 14 weeks high fat feeding to induce obesity and insulin resistance as confirmed by hyperinsulinaemia in these mice, the three groups of HFD mice (n = 6 per group) were administered either twice daily i.p. injections of saline vehicle (0.9% NaCl), Ex-4 or mGIP(3−30) (both at 25 nmol/kg body weight) for 21 days, with the final ND group also receiving daily saline vehicle injections. Power calculations were conducted based on changes of ileal hormone content between ND and HFD groups, with n = 6 considered appropriate to achieve significantly meaningful results. All experiments were conducted under the UK Animals (Scientific Procedures) Act 1986 & EU Directive 2010/63EU, approved by the UK Home Office under project licence PPL2902 and University of Ulster Animal Welfare and Ethical Review Body (AWERB).

2.3. Tissue processing

At the end of the treatment period, pancreatic and intestinal tissues were extracted from mice and fixed for 48 h in paraformaldehyde (4% w/v in phosphate buffered saline (PBS)) to preserve cellular architecture by cross-linking proteins. Tissues were then processed in an automated tissue processor which involved dehydrating in 70–100% ethanol followed by xylene immersion to remove wax before paraffin embedding. Embedded tissues were then cut at 5 μm sections on a microtome (Shandon Finesse 325, Thermo Scientific) and placed on poly-L-lysine coated slides [18].

2.4. Immunohistochemistry

To assess immunoreactive staining for insulin, glucagon, PYY, somatostatin, GLP-1, Ki-67 and TUNEL (Table 1), as appropriate, ileum and pancreatic sections were dewaxed in histoclear for 30 mins, before being rehydrated with decreasing concentrations of ethanol. Sections were blocked with 2.5% bovine serum albumin (BSA) and then incubated with designated primary antibody (Table 1) overnight. Importantly, the specificity of all primary antibodies for use in immunohistochemistry has been previously validated, and subsequently successfully employed in our laboratory [19,20]. On day 2, sections were then rinsed in PBS and incubated with suitable secondary antibody (Alexa Fluor® 594 for red and Alexa Fluor® 488 for green; Table 1) for 1 h at 37 °C. After a PBS wash, slides were then incubated with DAPI for 15 mins at 37 °C [18]. Finally, sections were mounted on coverslips using antifade mounting media before being viewed at 40x magnification using an Olympus IX51 inverted microscope and photographed using a DP70 digital camera system.

2.5. Image analysis

Image J software was used to assess total ileal crypt depth and villi length using the straight-line function. Total number of cells positive for GIP, GLP-1 and PYY, along with their counts in respective villi and crypt areas, were counted using the multi-point and polygon function. Cell^F software was used to analyse images for assessment of pancreatic islet, beta- and alpha-cell areas. For islet cell co-localisation studies, PPY and GLP-1 detection in alpha or somatostatin cells was determined by counting cells with positive PPY or GLP-1 and glucagon/somatostatin immunoreactivity and expressed as % of total alpha or somatostatin cells, as appropriate. For beta-cell proliferation, insulin and Ki67 positive cells were counted whereas for apoptosis, insulin and TUNEL positive cells were counted, as described previously [21].

2.6. Biochemical analysis

At the end of the study, non-fasting plasma glucose was directly measured from the cut tip on the tail vein of conscious mice using a hand-held Ascencia Contour blood glucose meter (Bayer Healthcare, Newbury, Berkshire, UK) at 10:00 h. For plasma insulin analysis, blood samples were collected into chilled fluoride/heparin glucose microcentrifuge tubes (Sarstedt, Numbrecht, Germany) and immediately centrifuged using a Beckman microcentrifuge (Beckman Instruments, Galway, Ireland) for 1 min at 13,000 *g* and stored at −20 °C, prior to determination of insulin concentrations by a modified insulin radioimmunoassay [22]. Intestinal tissues were then excised and immediately snap frozen in liquid nitrogen and stored at −80 °C. Subsequently, for

Table 1
Target, host and source of primary and secondary antibodies employed for immunofluorescent imaging experiments.

Primary antibodies			
Target	**Host**	**Dilution**	**Source**
Insulin	Mouse	1:500	Abcam, ab6995
Glucagon	Guinea pig	1:4	Raised in-house PCA2/4
PYY	Rabbit	1:500	Abcam, ab22663
GLP-1	Rabbit	1:4	Raised in-house XJIC8
SST	Rat	1:500	Biorad, 8330–009
GIP	Rabbit	1:4	RIC34/111 J, kindly donated by Professor L Morgan, Guildford, UK
Ki-67	Rabbit	1:200	Abcam, ab15580
Secondary antibodies			
Host and target	**Reactivity**	**Dilution**	**Fluorescent dilution and source**
Goat IgG	Mouse	1:500	Alexa Fluor 594, Invitrogen, UK
Goat IgG	Guinea pig	1:500	Alexa Fluor 488, Invitrogen, UK
Goat IgG	Rabbit	1:500	Alexa Fluor 594, Invitrogen, UK
Goat IgG	Rat	1:500	Alexa Fluor 488, Abcam

hormone content analysis tissues were homogenised using RIPA lysis buffer and 0.1% bovine lung protease inhibitor cocktail (Sigma-Aldrich, Dorset, UK). Homogenised tissues were centrifuged at 664 *g* for 20 mins at 4 °C, prior to analysis of total intestinal PYY (rat PYY ELISA, ORB441862-BOR, Stratech Scientific), GLP-1 (GLP-1 total ELISA, EZGLP-1 T-36 K, Millipore) and GIP (rat/mouse GIP ELISA, EZRM-GIP-55 K, Millipore) according to individual manufacturer's instructions. Total intestinal protein content of the samples was assessed using the Bradford protein assay.

2.7. Statistical analysis

GraphPad PRISM (version 5.0) software was used to perform statistical analysis. Values are expressed as mean ± S.E.M. Comparative analyses between groups were carried out using a one-way ANOVA with Bonferroni's post hoc test. There was no inclusion and exclusion criteria applied. Groups of data were considered to be significant if $p < 0.05$.

3. Results

3.1. Effects of sub-chronic Ex-4 and mGIP(3−30) treatment on metabolic parameters in HFD mice

As expected, the saline treated HFD group of mice exhibited a significant ($p < 0.01$) increase in body weight when compared to ND controls (Table 2). Body weight was decreased ($p < 0.05$) by both Ex-4 and mGIP(3−30) administration on day 21, that was related to reduced ($p < 0.05$) energy intake when compared to HFD control mice (Table 2). Blood glucose levels were decreased ($p < 0.05$) by Ex-4, but not mGIP (3−30), when compared to HFD control mice (Table 2). Terminal plasma insulin concentrations were increased ($p < 0.05$) in HFD mice, with Ex-4 significantly decreasing ($p < 0.05$) circulating insulin when compared to HFD controls (Table 2).

3.2. Effects of sub-chronic Ex-4 and mGIP(3−30) treatment on ileal morphology and gut hormone content in HFD mice

Crypt depth was decreased ($p < 0.001$) by HFD and restored to ND control levels by Ex-4, but not mGIP(3−30), intervention (Fig. 1A). Interestingly, both treatments increased ($p < 0.05$) ileum villi length when compared to HFD saline treated control mice (Fig. 1B). Representative images of ileum tissue from all groups of mice are provided within the supplementary material. HFD mice presented with decreased ($p < 0.05$–0.001) GLP-1, GIP and PYY ileal hormone content (Fig. 1C-E). Treatment with Ex-4 or mGIP(3−30) was able to fully restore GLP-1 ileal content to ND levels (Fig. 1D). However, neither intervention positively affect the reduced GIP or PYY ileum concentrations in HFD mice (Fig. 1C,E).

Table 2
Effects of Ex-4 and mGIP(3–30) on metabolic parameters after 21 days twice daily treatment in HFD female mice.

Diet/ Treatments	Body weight (g)	Blood glucose (mmol/l)	Plasma insulin (ng/ml)	Cumulative energy intake (KJ)
Normal diet (ND)	31.9 ± 1.1	7.9 ± 0.2	0.9 ± 0.2	1389.3 ± 31.4
High-fat diet (HFD)	47.4 ± 2.2**	8.6 ± 0.3	1.8 ± 0.3*	2785.5 ± 266.9***
HFD + Ex-4	40.6 ± 2.8$^{\Delta}$	7.6 ± 0.2$^{\Delta}$	0.5 ± 0.1$^{\Delta}$	2075.8 ± 250.7$^{\Delta}$
HFD + mGIP (3–30)	39.5 ± 1.3$^{\Delta}$	7.8 ± 0.3	1.8 ± 0.9	2132.5 ± 89.4$^{\Delta}$

Parameters were measured after 21 days twice daily treatment with Ex-4 and mGIP(3–30) (25 nmol/kg bw) in HFD female mice. ND and HFD mice received twice daily saline vehicle (0.9% NaCl) injections. Values are mean ± SEM (n = 6). *$p < 0.05$ and ***$p < 0.001$ compared to ND control mice. Δ$p < 0.05$ compared to saline treated HFD mice.

Figure legends

3.3. Effects of sub-chronic Ex-4 and mGIP(3−30) treatment on ileal GIP, GLP-1 and PYY cells in HFD mice

Fig. 2A, 3A & 4A depict representative images of ileal tissue from each treatment group stained for GIP, GLP-1 and PYY, respectively. HFD increased ($p < 0.05$) the number of GIP positive ileal cells as a whole, and specifically within crypts (Fig. 2B,C), but interestingly not in villi (Fig. 2D). Whilst mGIP(3−30) treatment had no obvious effect on these parameters, HFD mice treated with Ex-4 had similar levels of GIP positive ileal cells as ND controls when assessed per mm^2 of ileum (Fig. 2B), crypt (Fig. 2C) or villi (Fig. 2D). Numbers of GLP-1 positive ileal cells were not different between all groups of mice in the overall ileum and related crypts (Fig. 3B,C), however treatment with Ex-4 or mGIP(3−30) decreased ($p < 0.05$) numbers of GLP-1 positive villi cells whereas high fat feeding alone had no significant effect on this parameter (Fig. 3D). Total (Fig. 4B) and villi (Fig. 4D) numbers of PYY immunoreactive ileal cells were increased ($p < 0.01$) in HFD mice but returned to normal levels by both treatments, with no difference in overall and villi numbers of PPY positive cells in HFD mice treated with Ex-4 or mGIP(3−30) when comparted to ND controls (Fig. 4B,D). In addition, Ex-4 increased ($p < 0.05$) numbers of positively stained PYY cells in crypts when compared to ND mice (Fig. 4C), whereas all other groups of mice had similar levels of PYY immunoreactivity in ileal crypts (Fig. 4C).

3.4. Effects of sub-chronic Ex-4 and mGIP(3−30) treatment on pancreatic islet morphology in HFD mice

Saline and mGIP(3−30) treated HFD mice had increased ($p < 0.05$-$p < 0.01$, respectively) islet and beta-cell areas when compared to ND mice, whereas these factors were similar to ND controls in Ex-4 treated HFD mice (Fig. 5A,B). All groups of HFD mice presented with significantly ($p < 0.05$–0.01) elevated alpha-cell area (Fig. 5C). Islet PYY cell area remained unchanged across all groups of mice (Fig. 5D), whilst Ex-4 treatment increased ($p < 0.05$) delta-cell area when compared to ND controls (Fig. 5E). When viewed as a whole, the percentage of beta-cells was increased ($p < 0.01$) by mGIP(3−30) treatment (Fig. 5F), with a corresponding decrease ($p < 0.05$) in the percentage of alpha-cells (Fig. 5G). As expected, HFD mice had higher levels ($p < 0.01$) of glucagon positively stained cells within the centre of islets, which was unaffected by mGIP(3−30) but returned to ND levels by Ex-4 (Fig. 5H). In terms of beta-cell turnover, neither high fat feeding nor the treatment interventions had any significant impact on beta cell proliferation or apoptosis rates (Fig. 5I,J). Representative images of islets stained for insulin and glucagon from all groups of mice are shown within the supplementary material.

3.5. Effects of sub-chronic Ex-4 and mGIP(3−30) treatment on PYY and GLP-1 islet co-localisation in HFD mice

Representative images of pancreatic islets stained for PYY with somatostatin, as well as PYY or GLP-1 with glucagon, are shown in Fig. 6A. HFD increased ($p < 0.001$) co-localisation of PYY with glucagon, that was further augmented ($p < 0.01$) by both Ex-4 and mGIP(3−30) treatment (Fig. 6B). Whilst HFD decreased ($p < 0.01$) percentage co-staining of PYY with somatostatin when compared to ND mice, this effect was fully reversed by mGIP(3−30), but not Ex-4, treatment (Fig. 6C). High fat feeding did not alter co-immunoreactivity of GLP-1 and glucagon within islets, but both treatment interventions increased ($p < 0.05$–0.01) this hormone co-localisation when compared to ND mice (Fig. 6D).

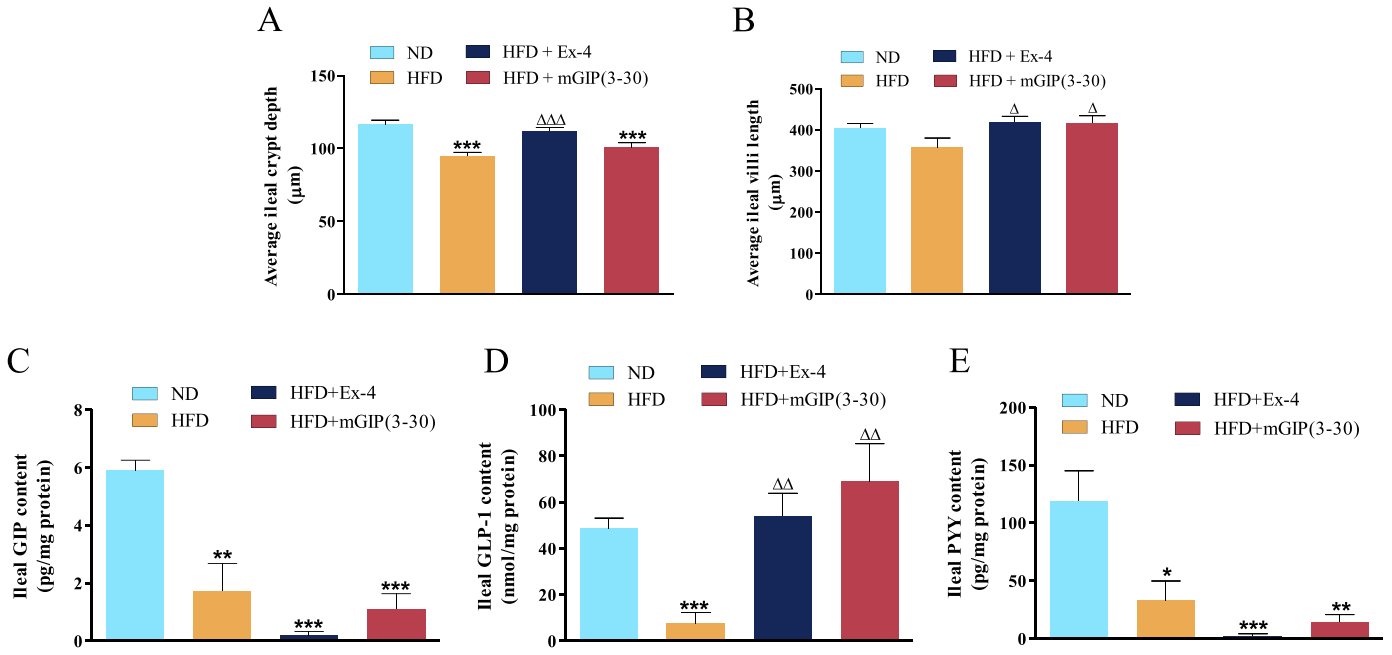

Fig. 1. Effects of Ex-4 and mGIP(3−30) on ileum morphology and gut hormone content. Parameters were measured after 21 days twice daily treatment with Ex-4 or mGIP(3−30) (each at 25 nmol/kg bw) in HFD female mice. Quantification of ileal (A) crypt depth and (B) villi length, (C) GIP content (pg/mg protein), (D) GLP-1 content (nmol/mg protein) and (E) PYY content (pg/mg protein). Values are mean ± SEM (n = 6). Approximately 100–120 crypts and villi were analysed per treatment group. *p < 0.05, **p < 0.01 and ***p < 0.001 compared to ND control mice. $^{\Delta}$p < 0.05, $^{\Delta\Delta}$p < 0.01 and $^{\Delta\Delta\Delta}$p < 0.001 compared to saline treated HFD mice.

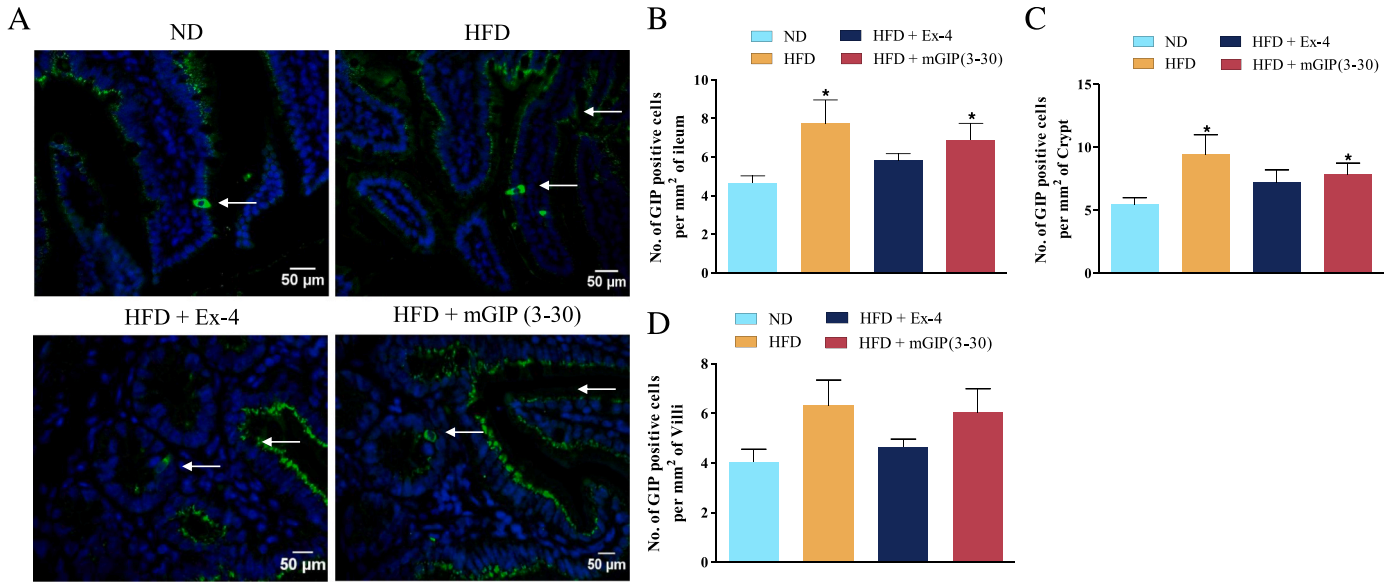

Fig. 2. Effect of Ex-4 and mGIP(3−30) on ileum GIP cell distribution. Parameters were measured after 21 days twice daily treatment with Ex-4 or mGIP(3−30) (each at 25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum stained for GIP (green) and DAPI (blue). Related quantification of (B) number of GIP positive cells per mm^2 of ileum, (C) number of GIP positive cells per mm^2 of crypt and (D) number of GIP positive cells per mm^2 of villi. White arrows indicate positively stained cells. Values are mean ± SEM (n = 6). *p < 0.05 compared to ND control mice.

4. Discussion

Obesity and related diabetes detrimentally alter the incretin hormone system, with compelling data to demonstrate reduced secretion of GLP-1 alongside excessive circulating GIP levels that subsequently leads to desensitisation of action [1]. These observations corroborate the benefits of sustained GLP-1R activation [23] or inhibition of GIPR signalling in obesity-diabetes in terms of body weight loss [24].

Importantly, whilst the anti-obesity effects of GLP-1R agonism or GIPR antagonism have been largely established in male mice [25,26], the current work confirms lack of sex-based dimorphic impact with either intervention. Effects on body weight appeared to be at least partly related to inhibition of appetite in both treatment groups, that is certainly characteristic of GLP-1R agonism [27,28]. Whilst there are recent reports of appetite suppressive effects of GIPR signalling upregulation [29,30] a GIPR antibody was demonstrated to inhibit food consumption in obese mice [31], whereas others report no effect on feeding following GIPR blockade in rodents [32]. In addition, GIPR KO

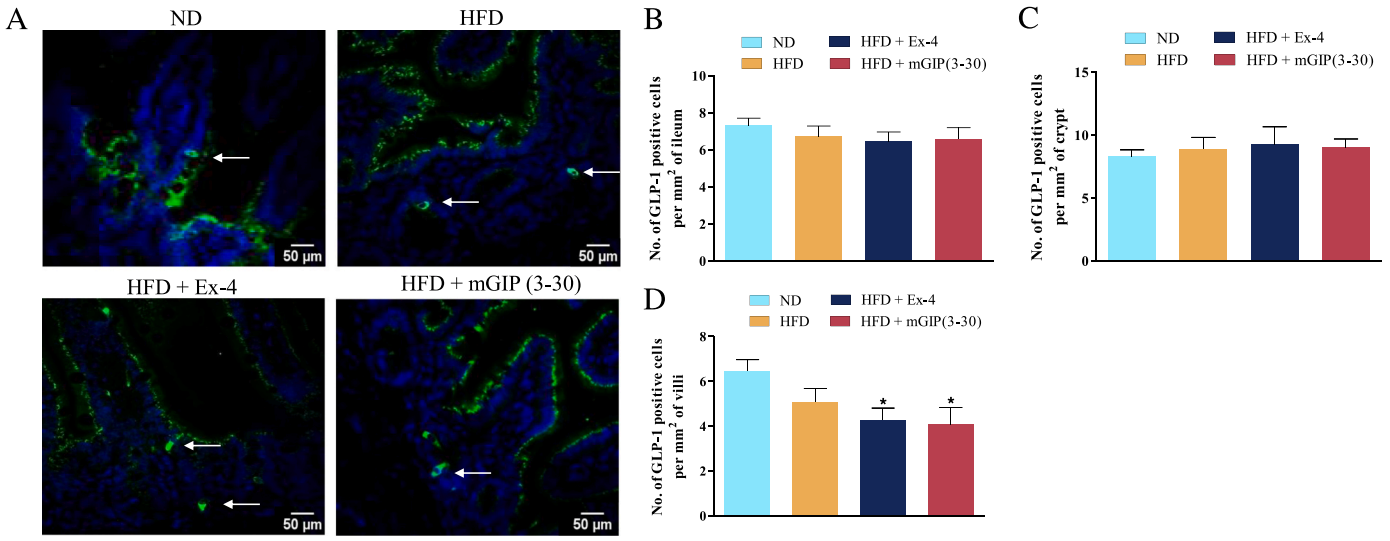

Fig. 3. Effect of Ex-4 and mGIP(3−30) on ileum GLP-1 cell distribution. Parameters were measured after 21 days twice daily treatment with Ex-4 or mGIP(3−30) (each at 25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum stained for GLP-1 (green) and DAPI (blue). Related quantification of (B) number of GLP-1 positive cells per mm^2 of ileum, (C) number of GLP-1 positive cells per mm^2 of crypt and (D) number of GLP-1 positive cells per mm^2 of villi. White arrows indicate positively stained cells. Values are mean ± SEM (n = 6). *$p < 0.05$ compared to ND control mice.

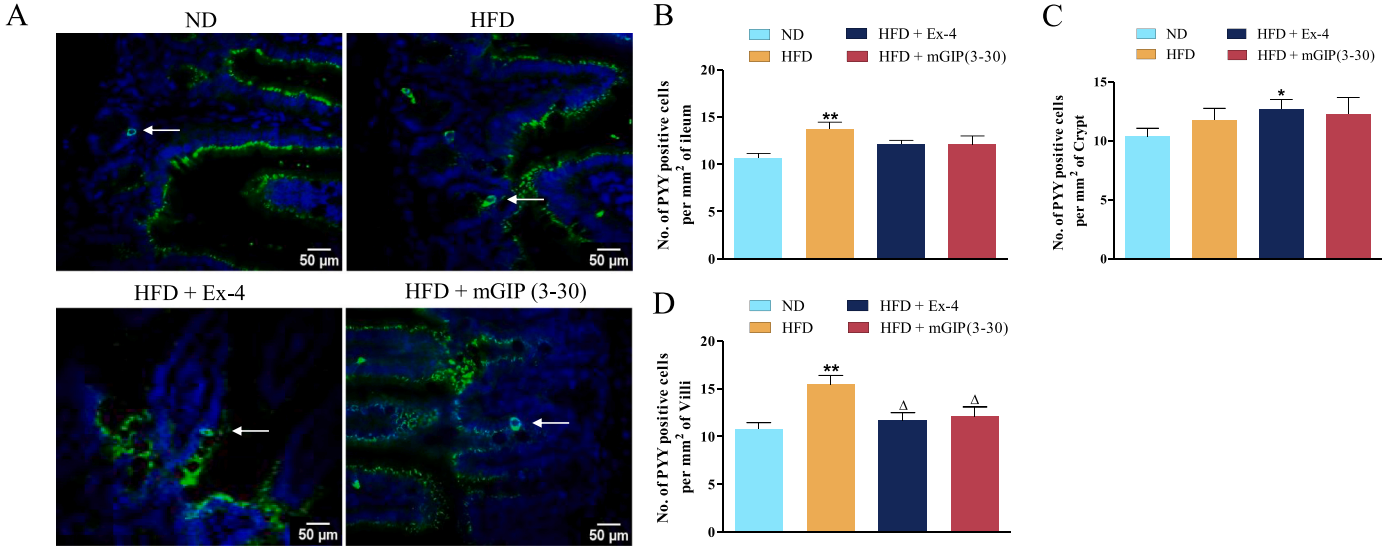

Fig. 4. Effect of Ex-4 and mGIP(3−30) on ileum PYY cell distribution. Parameters were measured after 21 days twice daily treatment with Ex-4 or mGIP(3−30) (each at 25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum stained for PYY (green) and DAPI (blue). Related quantification of (B) number of PYY positive cells per mm^2 of ileum, (C) number of PYY positive cells per mm^2 of crypt and (D) number of PYY positive cells per mm^2 of villi. White arrows indicate positively stained cells. Values are mean ± SEM (n = 6). *$p < 0.05$ and **$p < 0.01$ compared to ND control mice. $^{\Delta}p < 0.05$ compared to saline treated HFD control mice.

mice have been shown to present with both reduced [33] and unaffected [34,35] energy intake, further complicating matters. Given that the impact of mGIP(3−30) on decreasing energy intake was more prominent than Ex-4 in the current study, it is clear that further investigation into the effects of GIP on energy homeostasis is required. Indeed, multiple regulatory pathways and heterogeneity of central GIPR signaling has recently been noted in relation to GIP effects on appetite control [36]. Ex-4 marginally decreased circulating blood glucose, but mGIP(3−30) was without effect, which does correspond with changes in circulating insulin concentrations on day 21. This might imply improved insulin action in Ex-4, but not mGIP(3−30), treated HFD mice, but further assessment of insulin-induced cellular glucose uptake would be required to confirm this. Moreover, although non-fasting glucose values can be used as part of the criteria for diagnosing diabetes [37], it is not employed in isolation and also prone to meal induced fluctuations, which should also be considered when interpreting these data.

Obesity is known to have deleterious effects on the integrity and morphometry of intestinal EECs [38]. However, there is surprisingly limited knowledge on the influence of sustained GLP-1R agonism or GIPR antagonism on such factors. In the ileum of HFD mice, both Ex-4 and mGIP(3−30) treatment enlarged villi length indicating expansion of the absorptive surface, highlighting positive effects of both treatments on digestion, as has been previously observed for PYY(3−36) [39]. In good agreement with others, HFD prominently reduced crypt depth [40], an effect that was fully reversed by Ex-4, but not mGIP(3−30), therapy. This may suggest differential effects of the treatments on EEC secretions, or on production of new epithelial cells that will ultimately line crypts and villi [41]. However, such differences may also simply

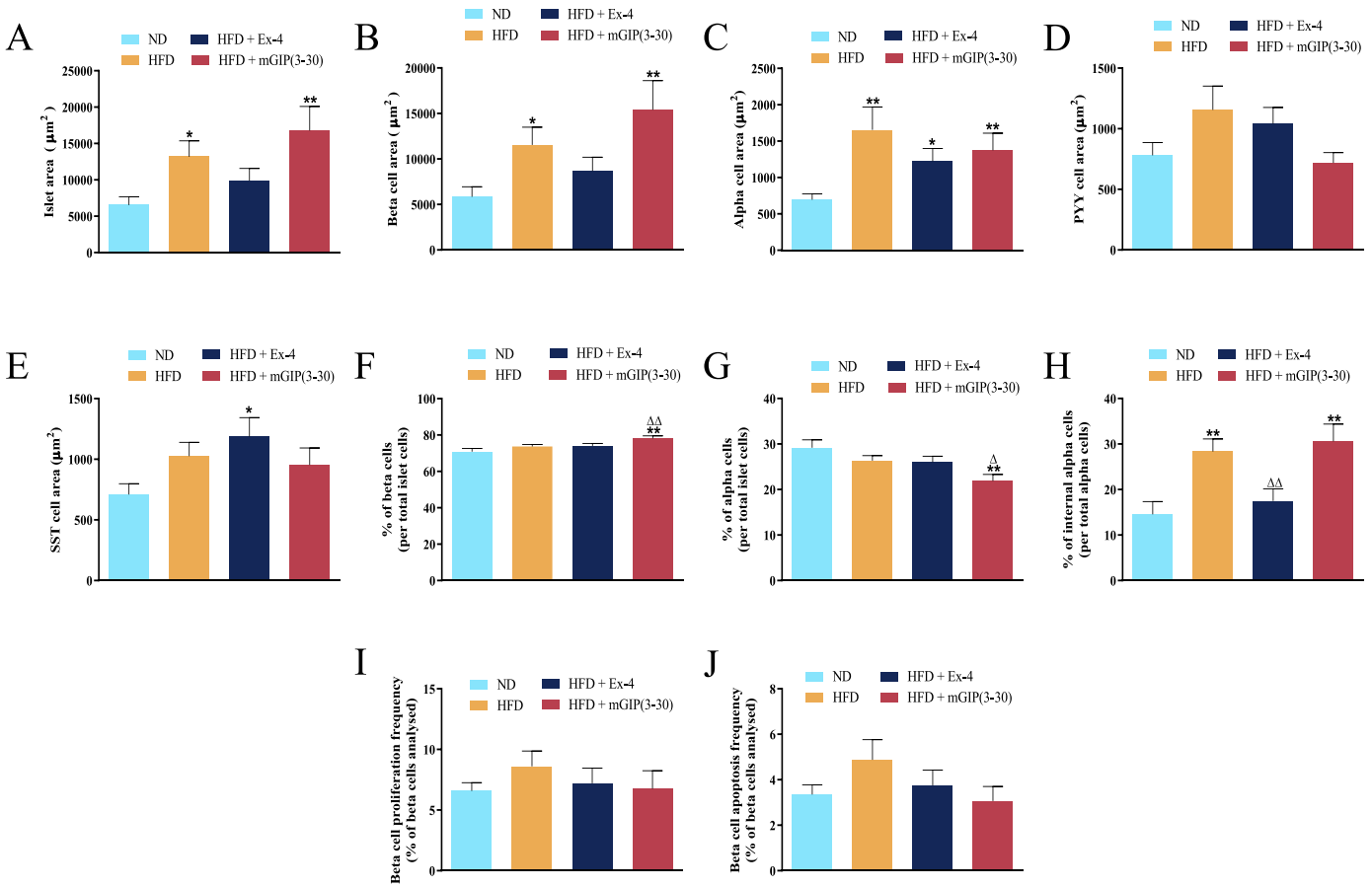

Fig. 5. Effect of Ex-4 and mGIP(3−30) on pancreatic islet morphology. Parameters were measured after 21 days twice daily treatment with Ex-4 or mGIP(3−30) (each at 25 nmol/kg bw) in HFD female mice. (A) Islet, (B) beta-cell, (C) alpha-cell, (D) PYY-cell, (E) delta-cell areas as well as (F) % of beta cell (per total islet cells), (G) % of alpha cells (per total islet cells), (H) % of internal alpha cells (per total alpha cells), (I) beta-cell proliferation frequency (% of beta cells analysed) and (J) beta-cell apoptosis frequency (% of beta cells analysed) were assessed. Values are mean ± SEM (n = 6). *$p < 0.05$ and **$p < 0.01$ compared to ND control mice. $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ compared to saline treated HFD mice.

reflect the very rapid turnover rate of EECs [42].

More in-depth investigation of ileal cell immunoreactivity revealed elevations of GIP and PYY cell populations in HFD mice, with no discernible impact on GLP-1 positive cell numbers. This is likely a reflection of the importance of PYY [43], and especially GIP [35,44,45], signalling on lipid metabolism, which would typically be more pronounced in HFD mice. Thus, GIP and PYY possess established biological actions to improve glucose homeostasis and satiety, respectively [46,47], and their elevated ileum immunoreactivity in HFD mice may represent an adaptive response to promote improved metabolic state in the face of sustained excess fat intake. Moreover, high fat feeding can lead to altered differentiation of EEC stem cells towards expression of more GIP positive cells [48]. mGIP(3−30) further enhanced GIP cell immunoreactivity within the ileum compared with ND mice, possibly as an adaptive response in the face of prolonged GIPR inhibition. Differences in the immunodetection of ileal GLP-1 and PYY in HFD mice is intriguing, given these hormones are thought to be co-localised and secreted from the same vesicles [49]. Thus, although the magnitude of alterations were relatively small, HFD mice had clear elevations in PYY, but not GLP-1, immunoreactive ileal cells. That said, both treatment options did concomitantly reduce GLP-1 and PYY cell populations within ileal villi. This is also notable considering that EEC L-cell density is thought to be greater in intestinal crypts than villi [50]. There was a small, but significant, increase in numbers of PYY positive cells in the crypts of Ex-4 treated HFD mice, which may merit further investigation. In that respect, EECs can modify hormone expression along the crypt-to-villus length [51], with upregulation of GLP-1R signalling potentially impacting this phenomenon.

Intriguingly, to the most part, alterations in EEC immunoreactivity were not matched by reciprocal changes in ileal hormone content, the difference presumably reflecting the balance between hormone synthesis and secretion. Thus, HFD mice presented with substantially reduced GIP, GLP-1 and PYY ileal content, that was not associated with reductions in ileal cell immunoreactivity of these hormones. Indeed, the number of GIP and PYY positive cells were increased in HFD mice, with no obvious change in GLP-1 cell immunoreactivity levels. Assessment of hormone plasma levels or intestinal expression may have helped with interpretation, although continual variations of hormone secretion with sustained excess dietary fat could be one likely explanation. As such, this would agree with the notion that prolonged high fat feeding alters the expression and secretory function of EECs [52], together with the inherent rapid turnover of these endocrine cells [42]. The only prominent effect of the treatment regimens in this regard was a noteworthy restoration of intestinal GLP-1 content. It follows that a significant part of the metabolic benefits of sustained Ex-4 or mGIP(3−30) administration in HFD mice is linked to enhanced GLP-1R mediated effects. Unfortunately, we were unable to measure circulating levels of GLP-1, that may have provided more information in this regard. However, in pancreatic islets we did detect increased co-localisation of GLP-1 within alpha-cells following treatment with either Ex-4 or mGIP(3−30), that would further support this notion. Although the current study focuses predominantly on GLP-1R activation and GIPR inhibition, it would be interesting to assess the impact of combined GLP-1R and GIPR activation on alterations of EEC populations in obese mice, especially given the

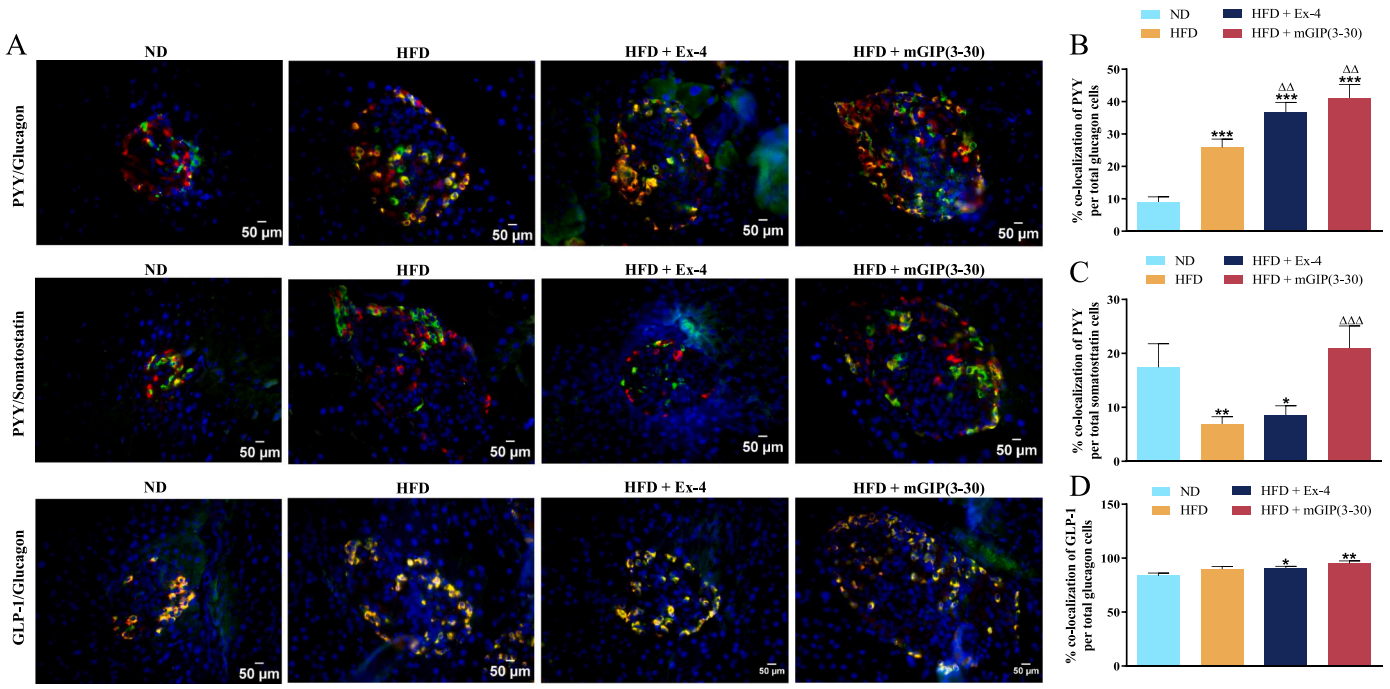

Fig. 6. Effect of Ex-4 and mGIP(3−30) on hormone co-localisation in islets. Parameters were measured after 21 days twice daily treatment with Ex-4 or mGIP(3−30) (each at 25 nmol/kg bw) in HFD female mice. (A) Representative images of islets stained for PYY/GLP-1 (red), glucagon/somatostatin (green) and DAPI (blue). Related quantification of % co-localisation of PYY with (B) glucagon or (C) somatostatin as well as (D) % co-localisation of GLP-1 with glucagon. Values are mean ± SEM (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 compared to ND control mice. $^{\Delta\Delta}$p < 0.01 and $^{\Delta\Delta\Delta}$p < 0.001 compared to saline treated HFD control mice.

relatively recent clinical approval of tirzepatide for obesity [53]. Furthermore, a dual acting GLP-1 agonist and GIP antagonist molecule has also been developed with body weight reducing effects in rodents and primates, and is now progressing towards phase II clinical trials [54].

As expected, HFD induced the classic enlargement of islet and beta-cell areas in mice [55]. Somewhat surprisingly, mGIP(3−30) treatment tended to enhance these islet adaptive responses as a result of increased numbers of beta-cells with a relative decrease in alpha-cells. This being despite the well characterised benefits of GIPR signalling activation to enhance beta-cell proliferation and protect against apoptosis [56]. Moreover, prolonged GIPR antagonism is believed to induce beta-cell rest and improve insulin action [57], that would be expected to reduce insulin demand. Yet, we did not detect any obvious effect of mGIP(3−30) on beta-cell turnover, perhaps indicating a potential effect on islet cell transdifferentiation and alteration of lineage of alpha- and beta-cells, that has been observed previously following modulation of GIPR signalling [58]. PYY is evidenced within islets [59], being linked to amelioration of beta-cell function and survival [60]. In that respect, it is interesting to note the pronounced elevations of detectable islet PYY in mGIP(3−30) treated HFD mice, in keeping with their augmented beta-cell area. Ex-4 treatment has a relatively modest impact on basic pancreatic islet histology in HFD mice. However, these mice did present with increased somatostatin staining in islets, implying that upregulation of GLP-1R signalling may possess more pronounced effects on intra-islet signalling pathways [61]. In good agreement, Ex-4 treated mice also had significantly reduced numbers of centrally located islet alpha-cells, with the characteristic alpha-cell halo in murine islets known to be particularly important for overall islet signalling and functionality [62]. In addition, effects may have been more pronounced with use of an extended treatment schedule, or increasing the doses of Ex-4 and mGIP(3−30). Finally, there is also a possibility that part of the observed treatment effects could be related, either directly or indirectly, to body weight loss. In this respect, pair-fed studies to mimic Ex-4 and mGIP(3−30) induced reductions of body weight would be required to help establish if adaptations of ileal and pancreatic islet morphology occur independently of body weight.

From a therapeutic viewpoint, our studies provide evidence that the established metabolic benefits of sustained GLP-1R activation or GIPR inhibition, through twice daily Ex-4 or mGIP(3−30) administration respectively, are linked to positive modulation of intestinal morphometry and hormone content. Thus, given that certain GLP-1R mimetics are already approved for obesity [63], an aspect of their clinical benefit is likely related to reversal of the detrimental effects of sustained excess calorie intake on EEC populations. Specifically, small alterations in EEC hormone detection following both treatment regimens results in a significant and consistent increase in ileal GLP-1 content. Our studies also demonstrate that mGIP(3−30) enhanced islet PYY levels, that may support the idea of elevated PYY signalling within pancreatic islets leading to an enhancement of beta-cell mass [60], but this still needs to be corroborated. However, the relative contribution of beneficial effects on ileal and pancreatic islet morphology towards the prominent metabolic advantages of approved GLP-1R mimetics, and GIPR antagonists in preclinical investigation for obesity, still needs to be fully determined. Moreover, aspects of these findings could also be relevant in terms of the exciting evolution of dual-acting drugs that concomitantly modulate GLP-1R and GIPR's [53,54].

Author contributions

AS, DK and RCM contributed to conduct and data collection of the study. AS, DK, RCM, NI and PRF contributed to study design, analysis and writing of the manuscript. All authors approved the final version of the manuscript.

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Declaration of Competing Interest

All authors declare no conflict of interest.

Data availability

The authors declare that the data supporting the findings of this study are available within the article. Any additional raw data supporting the conclusions of this article will be made available by the lead author (Ananyaa Sridhar, a.sridhar@ulster.ac.uk), without undue reservation.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.peptides.2023.171093.

References

[1] M.A. Nauck, D.R. Quast, J. Wefers, A.F. Pfeiffer, The evolving story of incretins (GIP and GLP-1) in metabolic and cardiovascular disease: a pathophysiological update, Diabetes Obes. Metab. 23 (2021) 5–29.

[2] N. Matikainen, L.H. Bogl, A. Hakkarainen, J. Lundbom, N. Lundbom, J. Kaprio, et al., GLP-1 responses are heritable and blunted in acquired obesity with high liver fat and insulin resistance, Diabetes Care 37 (2014) 242–251.

[3] J. Wang, Q. Wang, X. Yang, W. Yang, D. Li, J. Jin, et al., GLP– 1 receptor agonists for the treatment of obesity: role as a promising approach, Front. Endocrinol. 14 (2023) 1085799.

[4] N. Irwin, B.D. Green, V.A. Gault, R.S. Cassidy, F.P. O'Harte, P. Harriott, et al., Effects on glucose homeostasis and insulin secretion of long-term activation of the glucose-dependent insulinotropic polypeptide (GIP) receptor by N-AcGIP (LysPAL37) in normal mice, Peptides 27 (2006) 893–900.

[5] C.M. Martin, N. Irwin, P.R. Flatt, V.A. Gault, A novel acylated form of (d-Ala2) GIP with improved antidiabetic potential, lacking effect on body fat stores, Biochim. Biophys. Acta Gen. Subj. 1830 (2013) 3407–3413.

[6] I. Zaffina, M.C. Pelle, G. Armentaro, F. Giofrè, V. Cassano, A. Sciacqua, et al., Effect of dual glucose-dependent insulinotropic peptide/glucagon-like peptide-1 receptor agonist on weight loss in subjects with obesity, Front. Endocrinol. 14 (2023) 1095753.

[7] N. Irwin, P.L. McClean, K. Hunter, P.R. Flatt, Metabolic effects of sustained activation of the GLP-1 receptor alone and in combination with background GIP receptor antagonism in high fat–fed mice, Diabetes Obes. Metab. 11 (2009) 603–610.

[8] E.A. Killion, M. Chen, J.R. Falsey, G. Sivits, T. Hager, L. Atangan, et al., Chronic glucose-dependent insulinotropic polypeptide receptor (GIPR) agonism desensitizes adipocyte GIPR activity mimicking functional GIPR antagonism, Nat. Commun. 11 (2020) 4981.

[9] J.E. Campbell, Targeting the GIPR for obesity: to agonize or antagonize? Potential mechanisms, Mol. Metab. 46 (2021), 101139.

[10] V. Marks, GIP-the obesity hormone, in: W.P.T. James, S.W. Parker (Eds.), Current Approaches: Obesity, Duphar Laboratories Limited, London, 1988.

[11] K. Miyawaki, Y. Yamada, N. Ban, Y. Ihara, K. Tsukiyama, H. Zhou, et al., Inhibition of gastric inhibitory polypeptide signaling prevents obesity, Nat. Med. 8 (2002) 738–742.

[12] A.H. Sparre-Ulrich, L.S. Hansen, B. Svendsen, M. Christensen, F.K. Knop, B. Hartmann, et al., Species-specific action of (Pro3) GIP–A full agonist at human GIP receptors, but a partial agonist and competitive antagonist at rat and mouse GIP receptors, Br. J. Pharmacol. 173 (2016) 27–38.

[13] R.A. Perry, S.L. Craig, M.T. Ng, V.A. Gault, P.R. Flatt, N. Irwin, Characterisation of glucose-dependent insulinotropic polypeptide receptor antagonists in rodent pancreatic beta cells and mice, Clin. Med. Insight. Endocrinol. Diabetes 12 (2019), 1179551419875453.

[14] L.S. Gasbjerg, E.J. Bari, S. Stensen, B. Hoe, A.R. Lanng, D.S. Mathiesen, et al., Dose-dependent efficacy of the glucose-dependent insulinotropic polypeptide (GIP) receptor antagonist GIP (3–30) NH2 on GIP actions in humans, Diabetes Obes. Metab. 23 (2021) 68–74.

[15] F.M. Gribble, F. Reimann, Function and mechanisms of enteroendocrine cells and gut hormones in metabolism, Nat. Rev. Endocrinol. 15 (2019) 226–237.

[16] R. Dusaulcy, S. Handgraaf, S. Skarupelova, F. Visentin, C. Vesin, M. Heddad-Masson, et al., Functional and molecular adaptations of enteroendocrine L-cells in male obese mice are associated with preservation of pancreatic α-cell function and prevention of hyperglycemia, Endocrinology 157 (2016) 3832–3843.

[17] S.L. Craig, R.A. Perry, S.S. Vyavahare, M.T. Ng, V.A. Gault, P.R. Flatt, et al., A GIP/xenin hybrid in combination with exendin-4 improves metabolic status in db/db diabetic mice and promotes enduring antidiabetic benefits in high fat fed mice, Biochem. Pharmacol. 171 (2020), 113723.

[18] D. Khan, S. Vasu, R.C. Moffett, N. Irwin, P.R. Flatt, Influence of neuropeptide Y and pancreatic polypeptide on islet function and beta-cell survival, Biochim Biophys. Acta Gen. Subj. 1861 (2017) 749–758.

[19] S. Vasu, R.C. Moffett, B. Thorens, P.R. Flatt, Role of endogenous GLP-1 and GIP in beta cell compensatory responses to insulin resistance and cellular stress, PloS One 9 (2014), e101005.

[20] R.C. Moffett, S. Vasu, B. Thorens, D.J. Drucker, P.R. Flatt, Incretin receptor null mice reveal key role of GLP-1 but not GIP in pancreatic beta cell adaptation to pregnancy, PloS One 9 (2014), e96863.

[21] A. Sridhar, D. Khan, M. Abdelaal, J.A. Elliott, V. Naughton, P.R. Flatt, et al., Differential effects of RYGB surgery and best medical treatment for obesity-diabetes on intestinal and islet adaptations in obese-diabetic ZDSD rats, PloS One 17 (2022), e0274788.

[22] P.R. Flatt, C.J. Bailey, Plasma glucose and insulin responses to glucagon and arginine in Aston ob/ob mice: evidence for a selective defect in glucose-mediated insulin release, Horm. Metab. Res. 14 (1982) 127–130.

[23] N. Tanday, A. English, R.A. Lafferty, P.R. Flatt, N. Irwin, Benefits of sustained upregulated unimolecular GLP-1 and CCK receptor signalling in obesity-diabetes, Front. Endocrinol. 12 (2021), 674704.

[24] N. Irwin, V.A. Gault, F.P. O'Harte, P.R. Flatt, Blockade of gastric inhibitory polypeptide (GIP) action as a novel means of countering insulin resistance in the treatment of obesity-diabetes, Peptides 125 (2020), 170203.

[25] V. Pathak, V.A. Gault, P.R. Flatt, N. Irwin, Antagonism of gastric inhibitory polypeptide (GIP) by palmitoylation of GIP analogues with N-and C-terminal modifications improves obesity and metabolic control in high fat fed mice, Mol. Cell Endocrinol. 401 (2015) 120–129.

[26] B.D. Green, K.S. Lavery, N. Irwin, F.P. O'harte, P. Harriott, B. Greer, et al., Novel glucagon-like peptide-1 (GLP-1) analog (Val8) GLP-1 results in significant improvements of glucose tolerance and pancreatic β-cell function after 3-week daily administration in obese diabetic (ob/ob) mice, J. Pharmacol. Exp. Ther. 318 (2006) 914–921.

[27] K.A. Scott, T.H. Moran, The GLP-1 agonist exendin-4 reduces food intake in nonhuman primates through changes in meal size, Am. J. Physiol. Regul. Integr. Comp. Physiol. 293 (2007) R983–R987.

[28] S.E. Kanoski, S.M. Fortin, M. Arnold, H.J. Grill, M.R. Hayes, Peripheral and central GLP-1 receptor populations mediate the anorectic effects of peripherally administered GLP-1 receptor agonists, liraglutide and exendin-4, Endocrinol 152 (2011) 3103–3112.

[29] P.A. Mroz, B. Finan, V. Gelfanov, B. Yang, M.H. Tschöp, R.D. DiMarchi, et al., Optimized GIP analogs promote body weight lowering in mice through GIPR agonism not antagonism, Mol. Metab. 20 (2019) 51–62.

[30] Q. Zhang, C.T. Delessa, R. Augustin, M. Bakhti, G. Colldén, D.J. Drucker, et al., The glucose-dependent insulinotropic polypeptide (GIP) regulates body weight and food intake via CNS-GIPR signaling, Cell Metab. 33 (2021) 833–844.

[31] E.A. Killion, J. Wang, J. Yie, S.D. Shi, D. Bates, X. Min, et al., Anti-obesity effects of GIPR antagonists alone and in combination with GLP-1R agonists in preclinical models, Sci. Transl. Med. 10 (2018) eaat3392.

[32] T. Nakamura, H. Tanimoto, Y. Mizuno, M. Okamoto, M. Takeuchi, Y. Tsubamoto, et al., Gastric inhibitory polypeptide receptor antagonist, SKL-14959, suppressed body weight gain on diet-induced obesity mice, Obes. Sci. Pr. 4 (2018) 194–203.

[33] T. Hansotia, A. Maida, G. Flock, Y. Yamada, K. Tsukiyama, Y. Seino, et al., Extrapancreatic incretin receptors modulate glucose homeostasis, body weight, and energy expenditure, J. Clin. Investig. 117 (2007) 143–152.

[34] K. Miyawaki, Y. Yamada, N. Ban, Y. Ihara, K. Tsukiyama, H. Zhou, et al., Inhibition of gastric inhibitory polypeptide signaling prevents obesity, Nat. Med. 8 (2002) 738–742.

[35] G.A. Boer, S.N. Keenan, P.M. Miotto, J.J. Holst, M.J. Watt, GIP receptor deletion in mice confers resistance to high-fat diet-induced obesity via alterations in energy expenditure and adipose tissue lipid metabolism, Am. J. Physiol. Endocrinol. Metab. 320 (2021) E835–E845.

[36] A. Adriaenssens, J. Broichhagen, A. de Bray, J. Ast, A. Hasib, B. Jones, et al., Hypothalamic and brainstem glucose-dependent insulinotropic polypeptide receptor neurons employ distinct mechanisms to affect feeding, JCI Insight 8 (2023), e164921.

[37] H. Schneider, J. Shaw, P. Zimmet, Guidelines for the detection of diabetes mellitus-diagnostic criteria and rationale for screening, Clin. Biochem. Rev. 24 (2003) 77.

[38] A. Soares, E.J. Beraldi, P.E.B. Ferreira, R.B. Bazotte, N.C. Buttow, Intestinal and neuronal myenteric adaptations in the small intestine induced by a high-fat diet in mice, BMC Gastroenterol. 15 (2015) 1–9.

[39] A. Sridhar, D. Khan, P.R. Flatt, N. Irwin, R.C. Moffett, PYY (3-36) protects against high fat feeding induced changes of pancreatic islet and intestinal hormone content and morphometry, Biochim. Biophys. Acta Gen. Subj. 1867 (2023), 130359.

[40] Y. Xie, F. Ding, W. Di, Y. Lv, F. Xia, Y. Sheng, et al., Impact of a high-fat diet on intestinal stem cells and epithelial barrier function in middle-aged female mice, Mol. Med. Rep. 21 (2020) 1133–1144.

[41] V. Bonis, C. Rossell, H. Gehart, The intestinal epithelium–fluid fate and rigid structure from crypt bottom to villus tip, Front. Cell Dev. Biol. 9 (2021), 661931.

[42] F.M. Gribble, F. Reimann, Enteroendocrine cells: chemosensors in the intestinal epithelium, Annu. Rev. Physiol. 78 (2016) 277–299.

[43] Y. Shi, S. Lin, L. Castillo, A. Aljanova, R.F. Enriquez, A.D. Nguyen, et al., Peripheral-specific y2 receptor knockdown protects mice from high-fat diet-induced obesity, Obesity 19 (2011) 2137–2148.

[44] A. English, S.L. Craig, P.R. Flatt, N. Irwin, Individual and combined effects of GIP and xenin on differentiation, glucose uptake and lipolysis in 3T3-L1 adipocytes, Biol. Chem. 401 (2020) 1293–1303.

[45] S.K. Thondam, D.J. Cuthbertson, J.P. Wilding, The influence of Glucose-dependent Insulinotropic Polypeptide (GIP) on human adipose tissue and fat metabolism: implications for obesity, type 2 diabetes and Non-Alcoholic Fatty Liver Disease (NAFLD), Peptides 125 (2020), 170208.

[46] D.W. Porter, N. Irwin, P.R. Flatt, C. Hölscher, V.A. Gault, Prolonged GIP receptor activation improves cognitive function, hippocampal synaptic plasticity and glucose homeostasis in high-fat fed mice, Eur. J. Pharmacol. 650 (2011) 688–693.
[47] E. Karra, K. Chandarana, R.L. Batterham, The role of peptide YY in appetite regulation and obesity, J. Physiol. 587 (Pt 1) (2009) 19–25, 2009.
[48] K. Suzuki, N. Harada, S. Yamane, Y. Nakamura, K. Sasaki, D. Nasteska, et al., Transcriptional regulatory factor X6 (Rfx6) increases gastric inhibitory polypeptide (GIP) expression in enteroendocrine K-cells and is involved in GIP hypersecretion in high fat diet-induced obesity, J. Biol. Chem. 288 (2013) 1929–1938.
[49] A.M. Habib, P. Richards, G.J. Rogers, F. Reimann, F.M. Gribble, Co-localisation and secretion of glucagon-like peptide 1 and peptide YY from primary cultured human L cells, Diabetologia 56 (2013) 1413–1416.
[50] K. Kampmann, S. Ueberberg, B.A. Menge, T.G. Breuer, W. Uhl, A. Tannapfel, et al., Abundance and turnover of GLP-1 producing L-cells in ileal mucosa are not different in patients with and without type 2 diabetes, Metab. Clin. Exp. 65 (2016) 84–91.
[51] J. Beumer, B. Artegiani, Y. Post, F. Reimann, F. Gribble, T.N. Nguyen, et al., Enteroendocrine cells switch hormone expression along the crypt-to-villus BMP signalling gradient, Nat. Cell Biol. 20 (2018) 909–916.
[52] L. Ye, O. Mueller, J. Bagwell, M. Bagnat, R.A. Liddle, J.F. Rawls, High fat diet induces microbiota-dependent silencing of enteroendocrine cells, Elife 8 (2019), e48479.
[53] A.M. Jastreboff, L.J. Aronne, N.N. Ahmad, S. Wharton, L. Connery, B. Alves, et al., Tirzepatide once weekly for the treatment of obesity, N. Engl. J. Med. 387 (2022) 205–216.
[54] S. Lu, M. Chen, L. Atangan, E.A. Killion, R. Komorowski, Y. Cheng, et al., GIPR antagonist antibodies conjugated to GLP-1 peptide are bispecific molecules that decrease weight in obese mice and monkeys, Cell Rep. Med 2 (5) (2021), 100263.
[55] J. Ahrén, B. Ahrén, N. Wierup, Increased β-cell volume in mice fed a high-fat diet: a dynamic study over 12 months, Islets 2 (2010) 353–356.
[56] N. Irwin, P.R. Flatt, New perspectives on exploitation of incretin peptides for the treatment of diabetes and related disorders, World J. Diabetes 6 (2015) 1285.
[57] V. Pathak, S. Vasu, V.A. Gault, P.R. Flatt, N. Irwin, Sequential induction of beta cell rest and stimulation using stable GIP inhibitor and GLP-1 mimetic peptides improves metabolic control in C57BL/KsJ db/db mice, Diabetologia 58 (2015) 2144–2153.
[58] D. Sarnobat, R.C. Moffett, V.A. Gault, N. Tanday, F. Reimann, F.M. Gribble, et al., Effects of long-acting GIP, xenin and oxyntomodulin peptide analogues on alpha-cell transdifferentiation in insulin-deficient diabetic GluCreERT2; ROSA26-eYFP mice, Peptides 125 (2020), 170205.
[59] D. Khan, S. Vasu, R.C. Moffett, N. Irwin, P.R. Flatt, Islet distribution of Peptide YY and its regulatory role in primary mouse islets and immortalised rodent and human beta-cell function and survival, Mol. Cell Endocrinol. 436 (2016) 102–113.
[60] R.A. Lafferty, P.R. Flatt, N. Irwin, Established and emerging roles peptide YY (PYY) and exploitation in obesity–diabetes, Curr. Opin. Endocrinol. Diabetes Obes. 28 (2021) 253–261.
[61] S.A. Campbell, J. Johnson, P.E. Light, Evidence for the existence and potential roles of intra-islet glucagon-like peptide-1, Islets 13 (2021) 32–50.
[62] M.M. Holter, M. Saikia, B.P. Cummings, Alpha-cell paracrine signaling in the regulation of beta-cell insulin secretion, Front. Endocrinol. 13 (2022), 934775.
[63] J. Wang, Q. Wang, X. Yang, W. Yang, D. Li, J. Jin, et al., GLP− 1 receptor agonists for the treatment of obesity: role as a promising approach, Front. Endocrinol. 14 (2023) 1085799.