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PYY (3-36) protects against high fat feeding induced changes of pancreatic islet and intestinal hormone content and morphometry

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ABSTRACT

Background: Prolonged high fat feeding negatively impacts pancreatic and intestinal morphology. In this regard, direct effects of PYY(3–36) on intestinal cell and pancreatic islet morphometry are yet to be fully explored in the setting of obesity.
Methods: We examined the influence of 21-days twice daily treatment with PYY(3–36) on these parameters in mice fed a high fat diet (HFD).
Results: PYY(3–36) treatment decreased food intake, body weight and circulating glucose in HFD mice. In terms of intestinal morphology, crypt depth was restored to control levels by PYY(3–36), with an additional enlargement of villi length. PYY(3–36) also reversed HFD-induced decreases of ileal PYY, and especially GLP-1, content. HFD increased numbers of PYY and GIP positive ileal cells, with PYY(3–36) fully reversing the effect on PYY cell detection. There were no obvious differences in the overall number of GLP-1 positive ileal cells in all mice, barring PYY(3–36) marginally decreasing GLP-1 villi cell immunoreactivity. Within pancreatic islets, PYY(3–36) significantly decreased alpha-cell area, whilst islet, beta-, PYY- and delta-cell areas remained unchanged. However, PYY(3–36) increased the percentage of beta-cells while also reducing percentage alpha-cell area. This was related to PYY(3-36)-induced reductions of beta-cell proliferation and apoptosis frequencies. Co-localisation of islet PYY with glucagon or somatostatin was elevated by PYY(3–36), with GLP-1/glucagon co-visualisation increased when compared to lean controls.
Conclusion: PYY(3–36) exerts protective effects on pancreatic and intestinal morphology in HFD mice linked to elevated ileal GLP-1 content.
General significance: These observations highlight mechanisms linked to the metabolic and weight reducing benefits of PYY(3–36).

1. Introduction

Development of obesity involves unfavourable modulation of various central and peripheral signalling pathways directly related to the control of energy homeostasis [1]. Gastrointestinal (GIT) derived hormones appear to be critical mediators in this regard, highlighted by the recent clinical approval of glucagon-like peptide-1 (GLP-1) mimetics for obesity [2], with other related GIT hormone-based molecules likely to soon follow [3]. As well as GLP-1, peptide tyrosine (PYY) has emerged as an important regulator of energy intake through a direct inhibitory effect on hypothalamic hunger circuits [4]. Released postprandially from same intestinal L-cells as GLP-1, PYY is secreted as a 36-amino-acid residue peptide hormone [5]. The parent PYY peptide is then rapidly degraded by dipeptidyl peptidase-4 (DPP-4) in the circulation, to yield PYY(3–36) that exerts classical anorexigenic actions through specific activation of hypothalamic NPY2 receptors [6]. As such, the anti-obesity effects of PYY(3–36) or related NPY2 receptor agonists have been demonstrated in several rodent studies [7,8], with clear appetite suppressive actions also confirmed in humans [9]. Although the initial therapeutic optimism for PYY(3–36) based therapies was slightly tempered due to a relatively severe GIT-related adverse effect profile in man [10], a zinc-based extended-release drug formulation may go some way to alleviating these side-effects [11]. Furthermore, combined PYY (3–36) administration alongside GLP-1 and oxyntomodulin may help improve tolerability in humans, by allowing for reduced peptide doses whilst still exerting favourable effects on food intake and body weight [12], collectively supporting further development of PYY(3–36) preparations for obesity.

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To this end, it is imperative to understand the full consequences of sustained PYY(3–36) therapy in obesity-diabetes, a concept further substantiated by suggestion that the well-documented beneficial metabolic effects of Roux-en-Y gastric bypass (RYGB) are partly mediated by elevated intestinal and pancreatic PYY levels [13–16]. As such, pancreatic and enteroendocrine cellular adaptations such as an initial increase of beta-cell mass or reduction in functional enteroendocrine cell (EEC) numbers are key features in the development of obesity and diabetes [17,18]. Despite NPY2 receptor modulation being considered more therapeutically applicable for obesity rather than diabetes and evidence that the NPY1 receptor may represent the most predominant NPY receptor subtype in pancreatic islets [19], treatment with PYY (3–36) has also been shown to improve beta-cell health, either directly or indirectly [20–22].

Therefore, the aim of the current study was to explore endocrine mechanisms involved in improved metabolic state following sustained PYY(3–36) administration in mice fed a high fat diet (HFD). Specifically, this included investigation of alterations in intestinal morphology and gut cell hormone immunoreactivity profiles, as well as pancreatic islet architecture. Since PYY is synthesised at higher concentrations in the distal intestine [23], we assessed morphological changes and enteroendocrine cell (EEC) detection levels of GLP-1, GIP and PYY in the ileum of HFD mice.

2. Methods

2.1. Animals

Female NIH Swiss 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 drinking water and standard rodent diet (10% fat, 30% protein and 60% carbohydrate; Trouw Nutrition, Northwich, UK). 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 which resulted in increased body weight and non-fasting blood glucose concentrations (Table 1). Following this, two groups of HFD mice ($n = 6$) were administered twice daily i.p. injections of saline vehicle (0.9% NaCl) or PYY(3–36) (25 nmol/kg body weight, Synpeptide Co. Ltd., Shanghai) for 21 days with a separate saline treated normal diet (ND) group of mice ($n = 6$) employed as controls, equating to 18 experimental mice in total. All experiments were conducted under the UK Animals (Scientific Procedures) Act 1986 & EU Directive 2010/63EU as well as the UK Home Office animal project licence number PPL2902 and approved by the University of Ulster Animal Welfare and Ethical Review Body (AWERB).

2.2. Tissue processing

Pancreatic and distal ileal tissues were extracted from mice after 21 days treatment and fixed for 48 h in paraformaldehyde (4% *w/v* in phosphate buffered saline (PBS)) to preserve cellular architecture. Tissues were then placed in an automated tissue processor, which involved dehydrating in 70% to 100% ethanol followed by xylene immersion to remove wax before paraffin embedding. Embedded tissues were then sliced (5 μm sections) and placed on poly-L-lysine coated slides [24].

Table 1
Effects of PYY(3–36) on metabolic parameters in HFD mice.

Diet/ Treatments	Body weight (g)	Non-fasting blood glucose (mmol/l)	Cumulative energy intake (KJ)
	Final	Final	Day 21
Normal diet (ND)	33.7 ± 1.4	7.8 ± 0.5	1389.2 ± 31.4
High-fat diet (HFD)	45.0 ± 1.1***	9.1 ± 0.3*	2430.5 ± 238.4***
HFD + PYY (3–36)	41.8 ± 2.1	7.7 ± 0.3 $^{\Delta\Delta}$	1773.7 ± 155.5$^{\Delta}$

Parameters were measured before, during or after, as appropriate, 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. Values are mean ± SEM ($n = 6$). *$p < 0.05$ and ***$p < 0.001$ compared to ND mice; $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ compared to HFD mice.

2.3. Immunohistochemistry

To assess immunoreactive staining for insulin, glucagon, PYY, somatostatin, GLP-1, Ki-67 and TUNEL as appropriate, distal ileal 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 2) overnight. Importantly, specificity of all primary antibodies has been confirmed using peptide blocking experiments and show no cross-reactivity with related peptide hormones [25], although assess total rather than processed peptide forms. On day 2, sections were rinsed in PBS and incubated with suitable secondary antibody (Alexa Fluor® 594 for red and Alexa Fluor® 488 for green; Table 2) for 1 h at 37 °C. After PBS wash, slides were then incubated with DAPI for 15 mins at 37 °C [24]. Finally, sections were mounted on coverslips using antifade mounting media before being viewed at 40× magnification using an Olympus IX51 inverted microscope and photographed using a DP70 digital camera system.

2.4. 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 assessed using the multi-point and polygon function. This was achieved by employing the closed polygon function in ImageJ to determine the individual area of the ileum and crypt in each image. The area of crypt was then subtracted from the area of the ileum (crypt + villi) to calculate villi area, with only fully intact villi and crypts analysed. Cells stained positively for each hormone were then counted. The total number of positive cells in ileum/crypt/villi was divided by their respective areas, to obtain the number of GIP, GLP-1 or PYY cells per mm^2. Cell^F software was used to analyse images to quantify islet area, beta- and alpha-cell area as well as percentage of peptide positive cells. For co-localisation studies, PPY and GLP-1 in alpha and/or somatostatin cells was determined by counting cells with PPY or GLP-1 and glucagon/somatostatin positive cells and expressed as % of total alpha or

Table 2
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	1:200	Raised in-house PCA2/4
PYY	Rabbit	1:500	Abcam, ab22663
GLP-1	Rabbit	1:200	Raised in-house XJIC8
SST	Rat	1:500	Bio-Rad, 8330–009
GIP	Rabbit	1:400	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 Flour 594, Invitrogen, UK
Goat IgG	Guinea pig	1:500	Alexa Flour 488, Invitrogen, UK
Goat IgG	Rabbit	1:500	Alexa Flour 594, Invitrogen, UK
Goat IgG	Rat	1:500	Alexa Flour 488, Abcam

somatostatin cells, as appropriate. For beta-cell proliferation, insulin and Ki-67 positive cells were counted whereas for apoptosis, insulin and TUNEL positive cells were counted, as described previously [13].

2.5. Biochemical analysis

Non-fasting blood glucose was directly measured from the cut tip on the tail vein of conscious mice at 10:00 h using a hand-held Ascencia Contour blood glucose meter (Bayer Healthcare, Newbury, Berkshire, UK). In addition to being processed for histology, intestinal tissues were excised and immediately snap frozen in liquid nitrogen and stored at −80 °C for ileal hormone content analysis. Subsequently, tissues were homogenised using RIPA lysis buffer and 0.1% protease inhibitor. 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, EZRMGIP-55 K, Millipore) according to individual manufacturer's instructions. Total intestinal protein content of the samples was assessed using the Bradford protein assay.

2.6. 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 *post hoc* correction for multiple comparisons. There was no inclusion and exclusion criteria applied. Groups of data are considered to be from different populations if $p < 0.05$.

3. Results

3.1. Effects on metabolic parameters

As expected, 21 days twice daily treatment with PYY(3–36) significantly ($p < 0.05$) decreased cumulative energy intake in HFD mice (Table 1). This was associated with an approximate 7% reduction in body weight in PYY(3–36) treated mice, with the HFD leading to an increase ($p < 0.001$) in body weight (Table 1). As expected, the pre-treatment body weights of the HFD saline and PYY(3–36) treated groups of mice were significantly ($p < 0.001$) elevated when compared to ND mice, with body weights of 49.8 ± 1.7, 51.2 ± 2.5 and 34.0 ± 1.4 g, respectively. Non-fasting blood glucose levels were marginally elevated by high fat feeding but retuned to values identical to lean control mice following PYY(3–36) treatment intervention (Table 1), although there may be some inherent variability when assessing glucose concentrations in the fed state. However, in good agreement, the initial blood non-fasting glucose concentrations of the ND, HFD and HFD PYY (3–36) treatment groups of mice were 7.9 ± 0.2, 8.5 ± 0.5 and 8.6. ± 0.3 mmol/l, respectively.

3.2. Effects on ileal morphology

Representative images of ileum tissue from ND, HFD as well as HFD mice treated for 21 days with PYY(3–36) are shown in Fig. 1A. Crypt depth was significantly ($p < 0.001$) decreased by high fat feeding but restored to normal lean control levels by PYY(3–36) (Fig. 1B). Interestingly, PYY(3–36) treatment also increased ($p < 0.05$ - $p < 0.001$) ileum villi length when compared to both ND and HFD saline treated control mice (Fig. 1C).

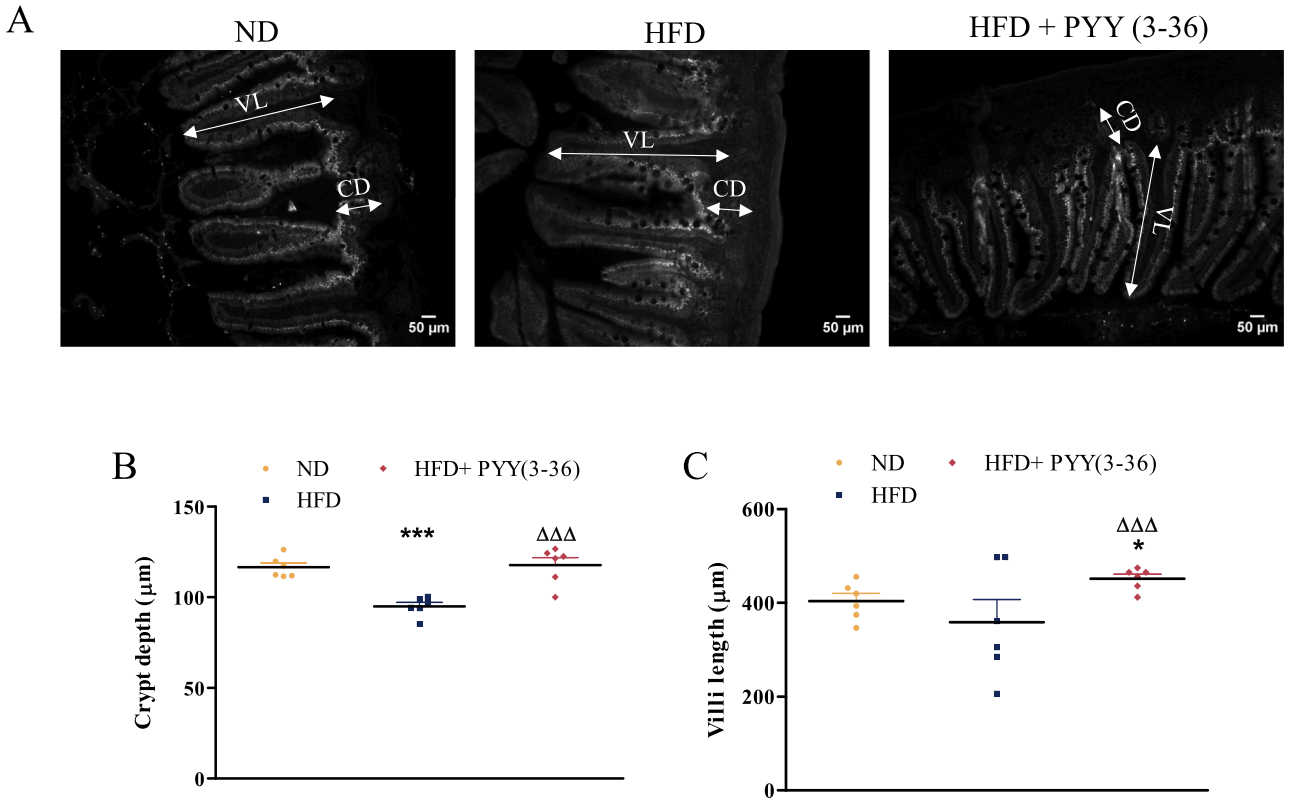

Fig. 1. Effects of PYY(3–36) on ileum morphology. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum where CD is crypt depth and VL is villi length. Related quantification of (B) crypt depth and (C) villi length. Values are mean ± SEM (n = 6). *p < 0.01 and ***p < 0.001 compared to ND control mice. $^{\Delta\Delta\Delta}$p < 0.001 compared to HFD mice.

3.3. Effects on ileal GIP, GLP-1 and PYY cells

Figs. 2A,3A&4A depict representative images of ileum from each treatment groups stained for GIP, GLP-1 and PYY, respectively. High fat feeding increased the total number of GIP positive ileal cells, but this just failed to reach significance (Fig. 2B). However, the number of GIP positive ileal cells in the crypt, but not within villus, was significantly increased ($p < 0.05$) in HFD mice (Fig. 2C,D). PYY(3–36) treatment had no significant impact on these parameters, but did tend to reduce numbers of GIP positive ileal cells (Fig. 2B-D). High fat feeding had no effect on the population of GLP-1 positive ileal cells (Fig. 3B-D), but concurrent PYY(3–36) administration decreased ($p < 0.05$) the number of GLP-1 positive villi cells when compared to lean control mice (Fig. 3D). The total number of PYY positive ileal cells was significantly increased ($p < 0.01$) in HFD mice but returned to normal levels by 21 days twice daily PYY(3–36) treatment (Fig. 4B). This effect was paralleled in the villi, but neither high fat feeding nor treatment intervention altered GLP-1 positive cell numbers in ileal crypts (Fig. 4C,D).

3.4. Effects on ileal hormone content

HFD mice presented with a significant ($p < 0.05$ - $p < 0.001$) decrease of GLP-1, GIP and PYY ileal hormone content (Fig. 5A-C). Treatment with PYY(3–36) mice was able to fully restore GLP-1 ileal content to ND levels in HFD mice, and PYY content was also not different from lean controls in these mice (Fig. 5B,C). However, PYY(3–36) had no impact on ileal GIP concentrations in HFD mice (Fig. 5A).

3.5. Effects on islet morphology

Representative images of islets stained for insulin and glucagon from all groups of mice are shown in Fig. 6A. As might be expected, HFD mice had increased ($p < 0.01$ - $p < 0.001$) islet, beta- and alpha-cells areas when compared to ND mice (Fig. 6B-D). PYY(3–36) reversed the impact of the HFD on alpha-cell area, but had no impact on total of beta-cell islet areas (Fig. 6B-D). However, the percentage of beta-cells was significantly ($p < 0.05$ - $p < 0.01$) increased by PYY(3–36) treatment when compared to either HFD or ND control mice (Fig. 6E). In good agreement, there was an associated significant ($p < 0.001$) decrease in the percentage of alpha-cells in PYY(3–36) treated mice (Fig. 6F). HFD mice also had increased ($p < 0.001$) central islet infiltration of alpha-cells, that was not affected by PYY(3–36) (Fig. 6G). Islet PYY and delta-cell areas remained unchanged across all groups of mice (Fig. 6H, I).

3.6. Effects on PYY and GLP-1 co-localisation in islets

Representative images of islets stained for PYY with glucagon or somatostatin as well as GLP-1 with glucagon are shown in Fig. 7A. PYY (3–36) treatment significantly ($p < 0.01$ and $p < 0.001$, respectively) increased the percentage co-detection of PYY with glucagon within islets when compared to both ND and HFD control mice (Fig. 7B). Whilst high fat feeding decreased ($p < 0.001$) percentage co-visualization of PYY with somatostatin, this effect was fully reversed by PYY(3–36) treatment (Fig. 7C). HFD mice had similar levels of islet co-detection of GLP-1 and glucagon as ND mice, but PYY(3–36) increased ($p < 0.05$) this cell population number when compared to lean control mice (Fig. 7D).

3.7. Effects on beta-cell turnover

Representative images of pancreatic islets stained for insulin and Ki-67 or TUNEL are shown in Fig. 8A. PYY(3–36) treatment significantly ($p < 0.05$) decreased beta-cell proliferation and apoptosis frequencies when compared to HFD mice, and beta-cell turnover levels where not different to ND control mice (Fig. 8B,C).

4. Discussion

In harmony with previous work [26–30], sub-chronic twice daily administration of PYY(3–36) resulted in a sustained appetite suppressive

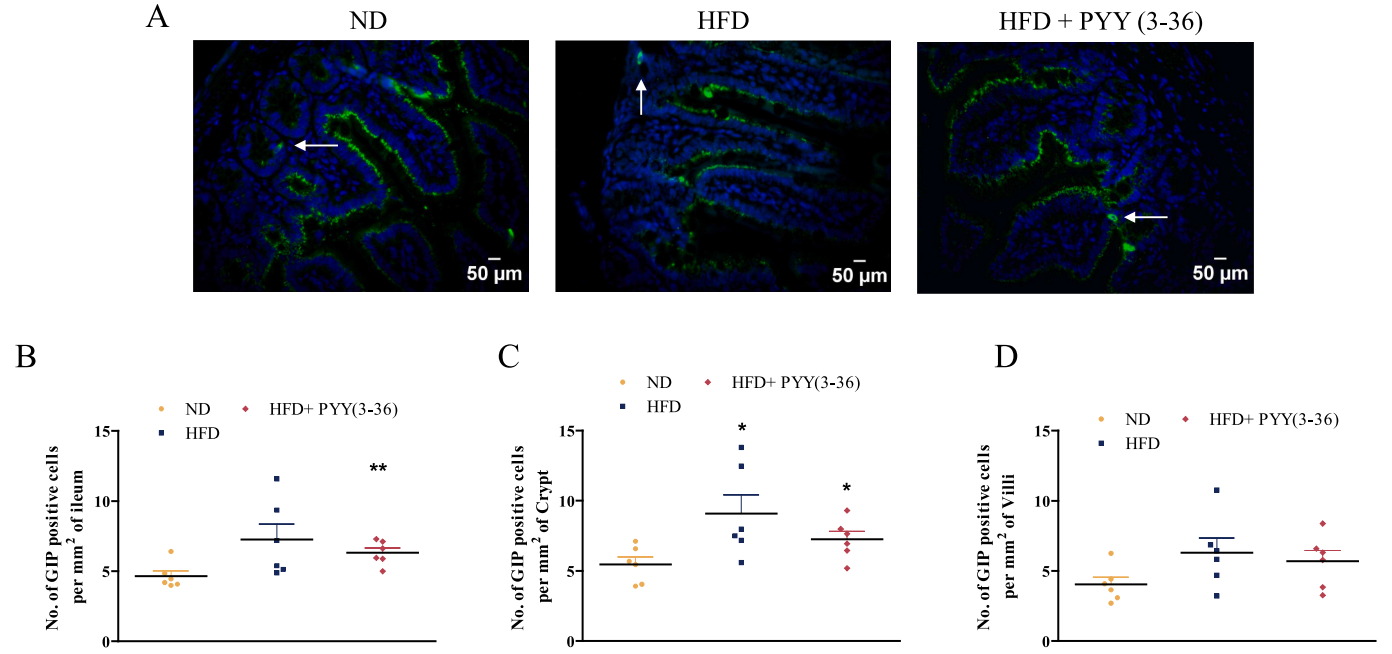

Fig. 2. Effect of PYY(3–36) on ileum GIP cell distribution. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum stained for GIP (green) and DAPI (blue). Supplementary Fig. 1 displays split channel as well as merged images of positively stained ileal GIP cells. 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, with 200–220 positively stained cells analysed. White arrows indicate positively stained cells. Values are mean ± SEM (n = 6). *p < 0.05 and **p < 0.01 compared to ND control mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

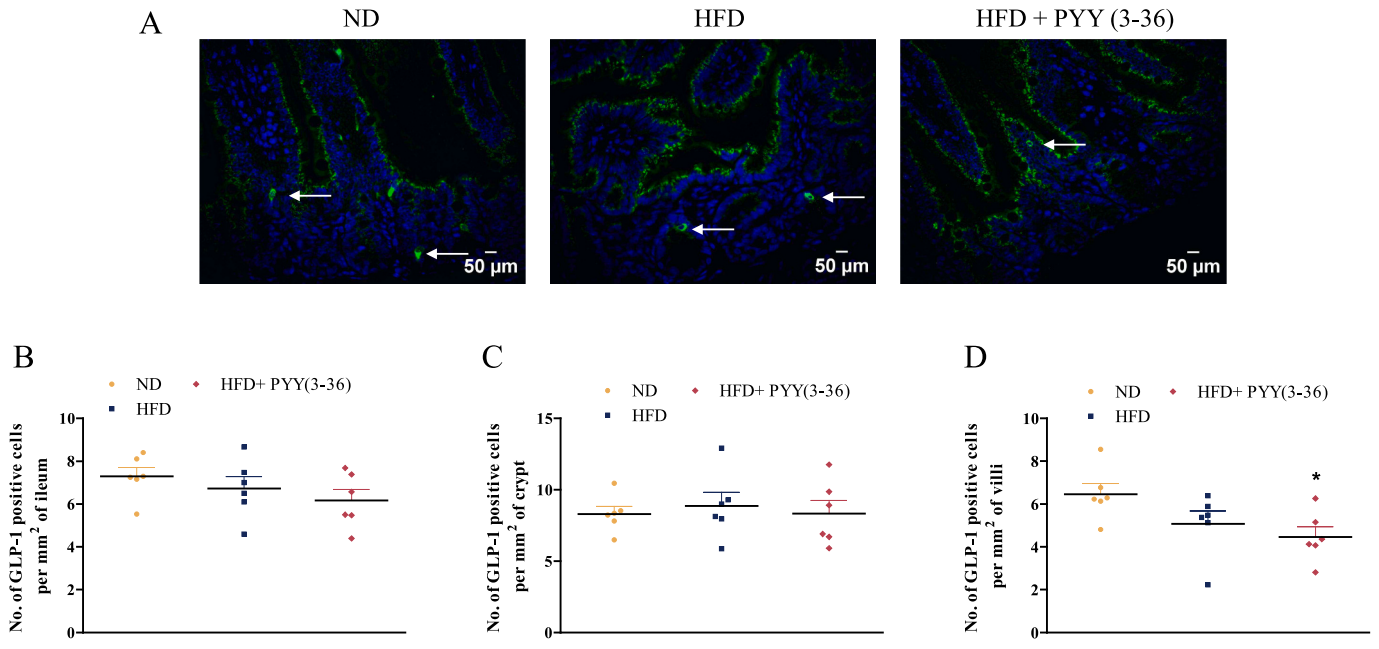

Fig. 3. Effect of PYY(3–36) on ileum GLP-1 cell distribution. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum stained for GLP-1 (green) and DAPI (blue). Supplementary Fig. 2 displays split channel as well as merged images of positively stained ileal GLP-1 cells. 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, with 200–220 positively stained cells analysed. White arrows indicate positively stained cells. Values are mean ± SEM (n = 6). *$p < 0.05$ compared to ND control mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

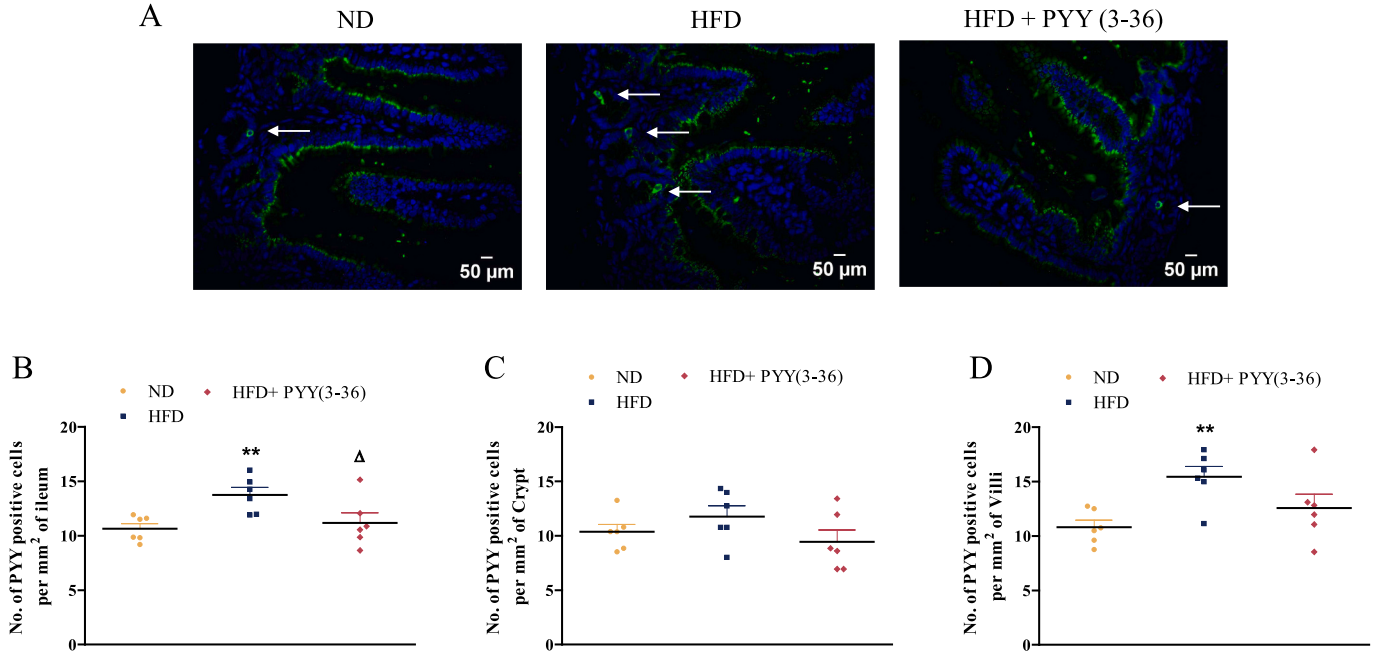

Fig. 4. Effect of PYY(3–36) on ileum PYY cell distribution. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of ileum stained for PYY (green) and DAPI (blue). Supplementary Fig. 3 displays split channel as well as merged images of positively stained ileal PYY cells. 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, with 200–220 positively stained cells analysed. White arrows indicate positively stained cells. Values are mean ± SEM (n = 6). **$p < 0.01$ compared to ND control mice. $^{\Delta}p < 0.05$ compared to HFD control mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

effect in HFD mice, which was accompanied by decreased body weight gain by the end of the treatment period. The possible induction of nausea may have contributed to the observed decreased food intake [10], but this is difficult to assess in mice. Body weight and food intake were not assessed during the 21-day treatment period, and we are therefore unable to comment on the evolution of PYY(3–36) induced effects on these parameters. Furthermore, while previous preclinical studies documenting the anti-obesity efficacy of PYY(3–36) have predominantly

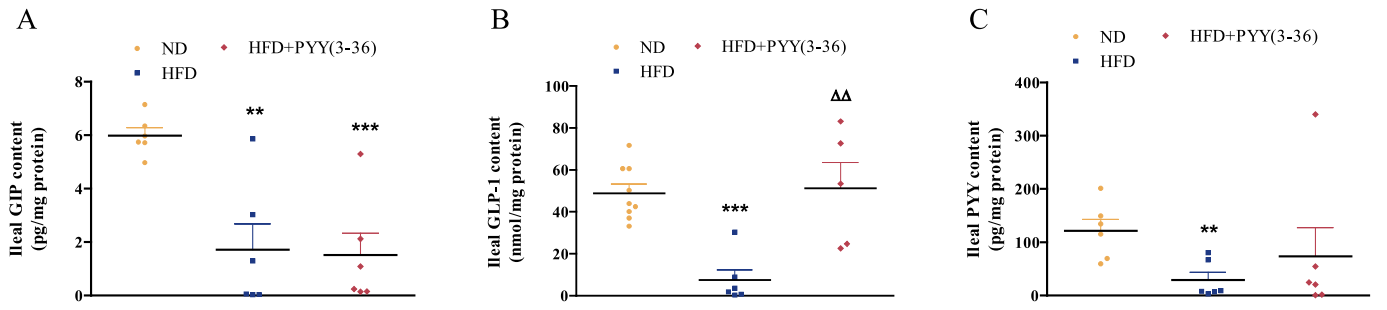

Fig. 5. Effect of PYY(3–36) on ileum gut hormone content. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. Ileal (A) GIP content (pg/mg protein), (B) GLP-1 content (nmol/mg protein) and (C) PYY content (pg/mg protein). Values are mean ± SEM (n = 6). *p < 0.05, **p < 0.01 and ***p < 0.001 compared to ND control mice. $^{\Delta\Delta}$p < 0.01 compared to HFD control mice.

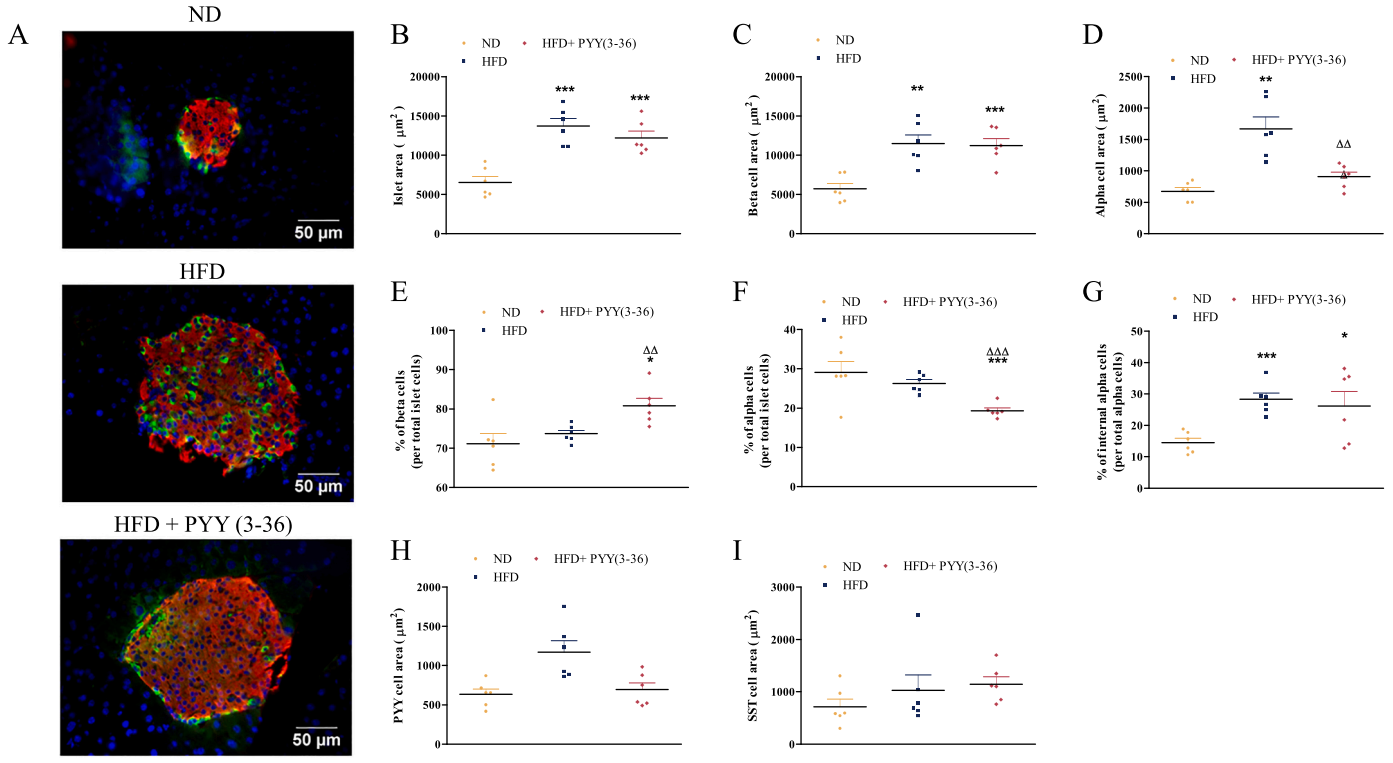

Fig. 6. Effect of PYY(3–36) on islet architecture and morphology. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of islets stained for insulin (red), glucagon (green) and DAPI (blue). Related quantification of (B) islet area, (C) beta-cell area, (D) alpha-cell area, (E) % of beta-cell (per total islet cells), (F) % of alpha-cells (per total islet cells), (G) % of internal alpha-cells (per total alpha-cells), (H) PYY cells area and (I) delta cell area. 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 HFD control mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

employed male rodents [4], the current work extends these findings to female mice. Thus, the ability of PYY(3–36) to inhibit appetite is not dependent on sex. Notably, this is in good agreement with observations in both female primates [31] and humans [12,32]. Considering the negative impact of obesity on reproductive function [33] and emerging evidence of a role for PYY in regulating female fertility [34–36], it is encouraging to note that benefits of PYY(3–36) on moderating energy intake are not sex dependent.

Despite knowledge that obesity can negatively affect the morphology and function of intestinal EECs [37], as well as predispose to type 2 diabetes and pancreatic islet dysfunction [38], the full impact of PYY (3–36) on these aspects has largely been overlooked in the literature. Indeed, observations in humans suggested a lack of effect of PYY(3–36) on pancreatic islet function [39], which was supported by evidence that the NPY2 receptor has low levels of expression within human and rodent islets [40]. However, it is evident that the NPY2 receptor is present on islets, where it likely exerts an insulinostatic effect to induce beta-cell rest [22]. This is supported in the current study through increased co-localisation of PYY with somatostatin in HFD mice treated with PYY (3–36). Thus, beta cell-rest has been confirmed to induce a protective effect against human beta-cells loss [41] as well as improving beta-cell glucose sensing allowing for chronically overstimulated beta-cells to replenish the immediately secretable insulin granule pool [42]. Accordingly, PYY(3–36) therapy increased the percentage of insulin producing beta-cells that was associated with reduced apoptosis and proliferation frequencies [22], whilst concurrently decreasing the percentage of alpha-cells.

Upregulated GLP-1 within alpha-cells is considered to be an adaptive consequence to help preserve islet morphology under conditions of metabolic stress, such as high fat feeding [43], with PYY(3–36) therapy augmenting this response alongside elevated alpha-cell PYY detection. Further to this, PYY(3–36) may also directly improve peripheral insulin

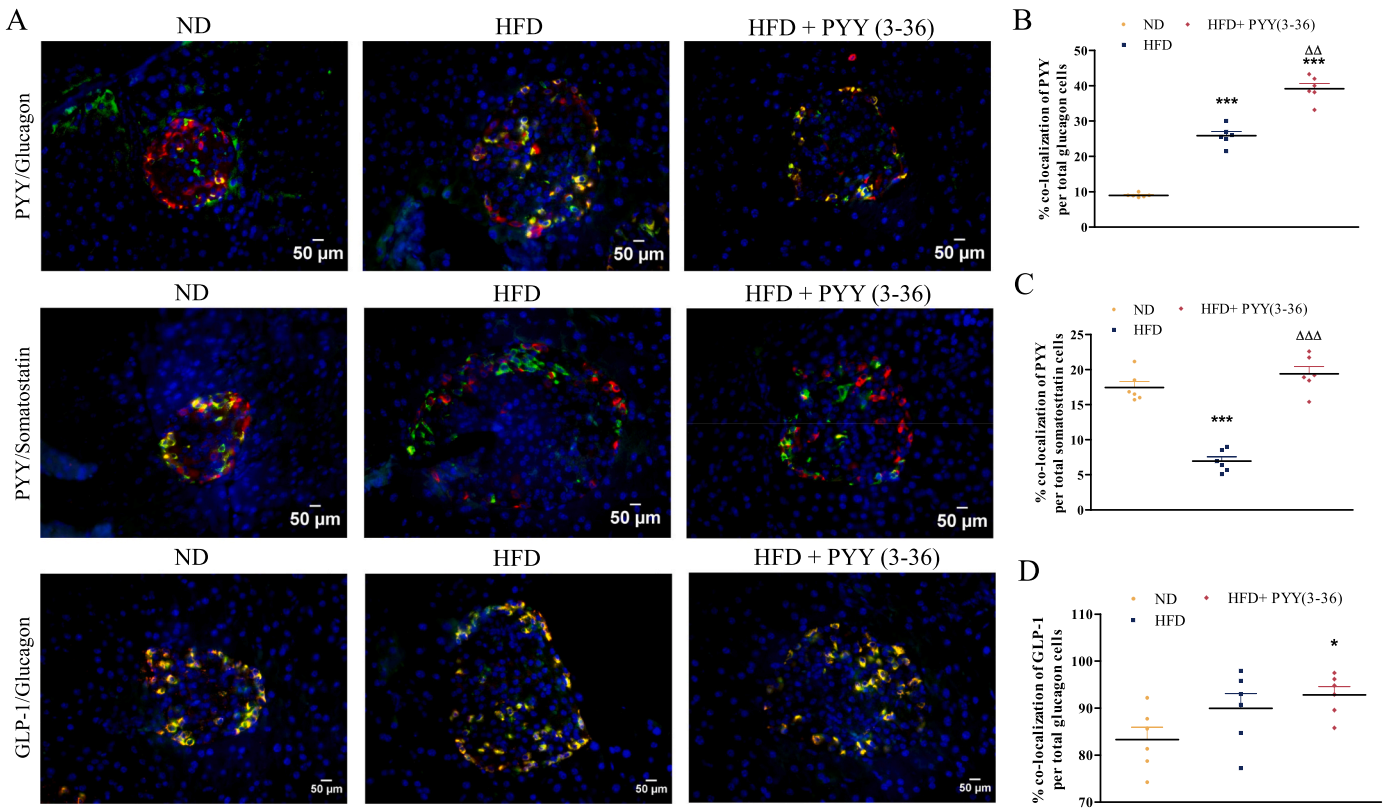

Fig. 7. Effect of PYY(3–36) on gut hormone co-localisation in islets. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of islets stained for PYY/GLP-1 (red), glucagon/somatostatin (green) and DAPI (blue). Related 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). *$p < 0.05$ and ***$p < 0.001$ compared to ND control mice. $^{\Delta\Delta}p < 0.01$ and $^{\Delta\Delta\Delta}p < 0.001$ compared to HFD control mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

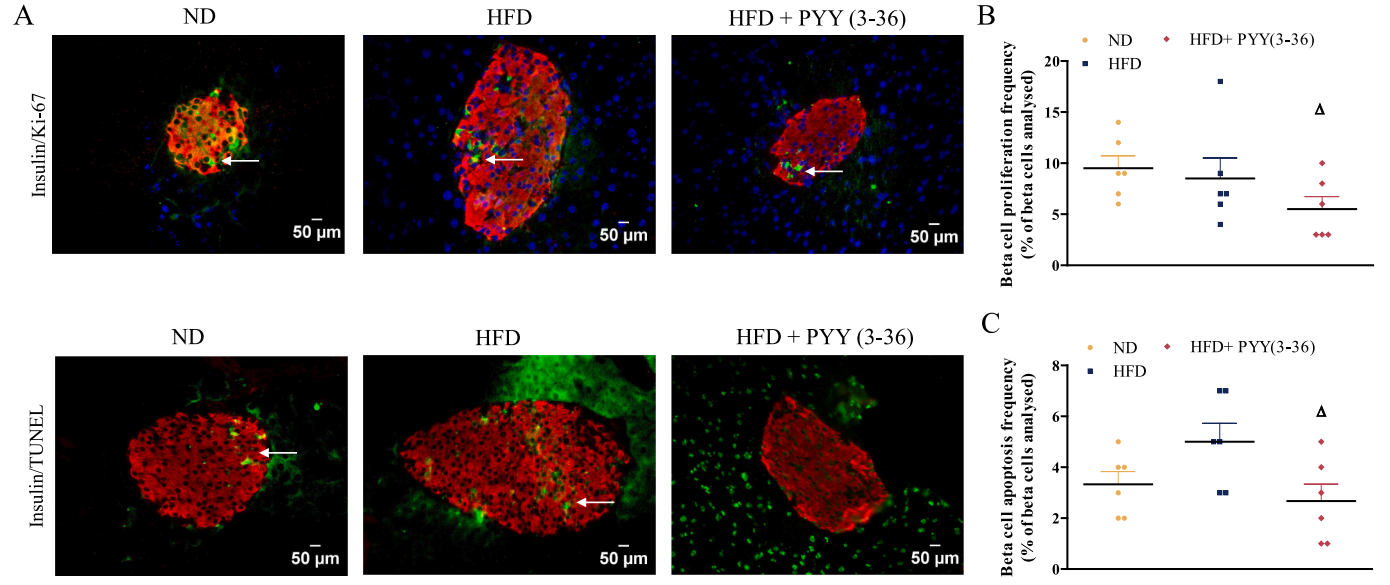

Fig. 8. Effect of PYY(3–36) on beta-cell growth and survival. Parameters were measured after 21 days twice daily treatment with PYY(3–36) (25 nmol/kg bw) in HFD female mice. (A) Representative images of islets stained for insulin (red) and Ki-67/TUNEL (green). Related quantification of (B) beta-cell proliferation frequency (% of beta-cells analysed) and (C) beta-cell apoptosis frequency (% of beta-cells analysed). Values are mean ± SEM (n = 6). $^{\Delta}p < 0.05$ compared to HFD control mice. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

sensitivity through mechanisms independent of body weight reductions [44]. Taken together, improved islet configuration following PYY(3–36) administration in female HFD mice leading to enhanced glucose homeostasis, alongside previous similar findings [20,45,46], reinforces a key role for PYY in preserving pancreatic intra-islet signalling and overall function.

Morphological investigation of the ileum in HFD mice revealed elongated crypts and villi following sustained PYY(3–36) treatment, indicating expansion of the absorptive surface. Although others have shown that HFD alone leads to increased crypt and villi area, this effect is not consistent throughout the length of the intestine [47–49]. In good agreement, Soares and co-workers documented that HFD decreased ileal crypt and villi length as observed in the current study, with a contrasting effect in the duodenum and jejunum [50], likely because of initial increased nutrient stimulation in these sections of the gut under high fat feeding. Moreover, HFD consumption can reduce the motility of the proximal intestine leading to fewer nutrients reaching the ileum [51], that also largely agrees with our findings.

More detailed morphological analysis of the ileum revealed that high fat feeding significantly increased the number of GIP secreting EECs. This is perhaps anticipated given that fat is a potent activator of pathways for GIP secretion [52]. Indeed, elevated digesta fat content within the ileum can trigger stem cell differentiation towards GIP positive cells within intestinal crypts [48,50]. Surprisingly, overall ileal GIP content was substantially reduced in HFD mice, with concurrent PYY(3–36) therapy having no obvious impact on this. However, such observations may also need to be considered in context of circulating GIP levels that unfortunately were not assessed in the present study. Indeed, somewhat of a conundrum exists in terms of the impact of GIP in obesity and diabetes. Thus, ablation of GIP signalling is linked to amelioration of spontaneous and diet-induced obesity and diabetes in rodents [53], yet activation of GIP receptors can decrease appetite [54] and exert clear anti-diabetic benefits [55,56].

Interestingly, there was a relatively small effect of HFD on ileal GLP-1 cell immunoreactivity, but this was accompanied by a quite dramatic reduction in intestinal GLP-1 content in these mice. This perhaps supports the notion that HFD can reduce expression of GLP-1 specific genes and impair secretory function in rodents [57]. Moreover, high fat feeding in zebrafish results in EEC silencing, that impairs nutrient sensing and signalling of these cells [58]. In general, alterations of hormone content were more obvious than related changes in numbers of positively stained intestinal cells, possibly suggesting a negative impact of HFD on the synthesis, storage and secretion of these hormones rather than directly on cell viability. In this regard it may have been interesting to assess intestinal expression levels of GLP-1, GIP and PYY, although there is an imperfect relationship between gene and protein expression, that would be further compounded by adaptations to prolonged HFD and the relatively rapid turnover of EECs [59]. It is also notable that previous investigations report a more prominent effect of HFD on colonic rather than ileal EECs [60,61], and in that respect direct comparison of the impact of both HFD and PYY(3–36) intervention on ileal and colonic intestinal tissue in the current setting would be interesting. More remarkable was the ability of PYY(3–36) treatment to restore intestinal GLP-1 content to normal levels in HFD mice, despite a small decrease in villi GLP-1 cell detection in these mice. In agreement, PYY (3–36) and associated NPY2 receptor activation has been demonstrated to augment nutrient-stimulated GLP-1 secretion [62]. In that respect it would have been interesting to assess circulating levels of GLP-1, PYY (3–36) and GIP in the current study to help confirm the suggestion, but unfortunately this was not possible. Indeed, there is a suggestion that GLP-1 and PYY(3–36) exert addictive benefits on glucose homeostasis and inhibition of food intake [63]. Furthermore, recent characterisation of highly effective GLP-1/NPY2 receptor hybrid peptides further support the notion of complementary biological action profiles between the GLP-1 and PYY(3–36) [64]. It is perhaps unsurprising that exogenous PYY (3–36) delivery reduced HFD-induced elevations of PYY positive cells within the ileum of mice. That said, PYY content within the ileum was decreased by HFD, but not with concomitant twice daily PYY(3–36) treatment. In that respect, GLP-1 and PYY have been shown to be co-localised within the same secretory vesicle pools in EECs and released in parallel [65], and thus increases of both GLP-1 and PYY content in PYY(3–36) treated HFD mice may reflect this phenomenon.

In summary, our findings provide evidence that PYY(3–36) administration exerts effects on hormone immunoreactivity levels within EECs in HFD mice, with substantial augmentation of ileal GLP-1 content. In addition, PYY(3–36) treatment directly improved pancreatic islet morphology and beta-cell turnover. Given the numerous well described anti-obesity and-diabetic actions of GLP-1 [66,67] alongside evidence of reciprocal actions between GLP-1 and PYY [68], it is likely that the metabolic and weight reducing benefits of PYY(3–36) are linked, in part, to upregulated GLP-1 receptor activity.

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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CRediT authorship contribution statement

A. Sridhar: Methodology, Validation, Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization. **D. Khan:** Methodology, Validation, Data curation, Formal analysis, Investigation, Writing – review & editing. **P.R. Flatt:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition. **N. Irwin:** Conceptualization, Methodology, Validation, Writing – original draft, Writing – review & editing. **R. C. Moffett:** Validation, Writing – review & editing, Visualization, Supervision, Project administration, Funding acquisition.

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 corresponding author, without undue reservation.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbagen.2023.130359.

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