Biochimica et Biophysica Acta 1850 (2015) 1206–1214

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbagen

Functional GIP receptors play a major role in islet compensatory response to high fat feeding in mice

R. Charlotte Moffett *[,1], Srividya Vasu [1], Peter R. Flatt

SAAD Centre for Pharmacy and Diabetes, University of Ulster, Cromore Road, Coleraine BT52 1SA, Northern Ireland, UK

ARTICLE INFO

Article history:
Received 27 October 2014
Received in revised form 30 January 2015
Accepted 9 February 2015
Available online 14 February 2015

Keywords:
Gastric inhibitory polypeptide
Alpha-cell
K-cell
Immunohistochemistry

ABSTRACT

Background: Consumption of high fat diet and insulin resistance induce significant changes in pancreatic islet morphology and function essential for maintenance of normal glucose homeostasis. We have used incretin receptor null mice to evaluate the role of gastric inhibitory polypeptide (GIP) in this adaptive response.
Methods: C57BL/6 and GIPRKO mice were fed high fat diet for 45 weeks from weaning. Changes of pancreatic islet morphology were assessed by immunohistochemistry. Body fat, glucose, insulin, glucagon, glucagon-like peptide 1 (GLP-1) and GIP were assessed by routine assays.
Results: Compared with normal diet controls, high fat fed C57BL/6 mice exhibited increased body fat, hyperinsulinaemia and insulin resistance, associated with decreased pancreatic glucagon, unchanged pancreatic GLP-1 and marked increases of insulin, islet number, islet size and both beta- and alpha-cell areas. Beta cell proliferation and apoptosis were increased under high fat feeding, but the overall effect favoured enhanced beta cell mass. A broadly similar pattern of change was observed in high fat fed GIPRKO mice but islet compensation was severely impaired in every respect. The inability to enhance beta cell proliferation was associated with the depletion of pancreatic GLP-1 and lack of hyperinsulinaemic response, resulting in non-fasting hyperglycaemia. GIP and GLP-1 were expressed in islets of all groups of mice but high fat fed GIPRKO mice displayed decreased numbers of GLP-1 containing alpha cells plus non-functional enhancement of pancreatic GIP content.
General significance: These data suggest that GIP released from islet alpha-cells and intestinal K-cells plays an important role in islet adaptations to high fat feeding.

1. Introduction

Obesity is an overwhelming health problem in many societies linked to sedentary lifestyle and consumption of energy rich high fat diets [1]. The accompanying induction of insulin resistance necessitates beta cell compensation to stave off the progression to hyperglycaemia, but when the capacity of beta cell adaptation is exceeded overt diabetes ensues. This scenario is well illustrated by the age-related progression of diabetes in relation to changes of islet morphology in C57BL/KsJ db/db mice [2]. Thus, in contrast to high fat fed wild-type controls, these animals rapidly succumb to beta cell exhaustion and destruction following short compensatory period of beta cell expansion and hyperactivity [3].

In the present study, we have used normal C57BL/6 mice together with GIPRKO mice on same genetic background to evaluate the role of glucose-dependent insulinotropic polypeptide (GIP) in islet compensation to prolonged high fat feeding. Although discovered many years ago and originally named gastric inhibitory polypeptide [4], GIP underwent a long period of being the 'neglected incretin' [5] overshadowed by the advent of molecular biology which led to the discovery and extensive characterisation of proglucagon-derived GLP-1 released from intestinal L-cells following cleavage of the precursor peptide by PC1/3 [6]. More recent studies have revisited GIP alongside GLP-1 [7,8] and indicate that both peptides have important physiological effects that can be exploited for treatment of diabetes using either GLP-1 mimetics or DPPIV inhibitors which enhance the bioactivity of both incretin peptides by inhibiting their rapid in vivo degradation [8,9].

Although GLP-1 has greater insulin-releasing effects at pharmacological concentrations [10], it is apparent that the higher circulating concentrations of GIP combined with more pronounced increase in secretion following feeding make GIP the major physiological incretin [7,11]. The peptide exerts pleiotropic actions on beta cells ranging from the stimulation of insulin biosynthesis and secretion to the enhancement of beta cell proliferation and protection against apoptosis induced by cytotoxic attack [7]. Interestingly GIP also has direct beneficial extra-pancreatic actions including positive effects on bone formation that may be physiologically important following feeding [12].

Recent studies on cellular biology of GIP and GLP-1 have indicated that both hormones are synthesised and secreted from islet alpha cells under conditions of cellular stress imposed by beta cytotoxic attack or increased insulin demand such as observed in pregnancy [13–15].

* Corresponding author. Tel.: +44 28 70324432.
E-mail address: s.vasu@ulster.ac.uk (R.C. Moffett).
[1] Contributed equally to this manuscript.

http://dx.doi.org/10.1016/j.bbagen.2015.02.006
0304-4165/

Thus it is commonly believed that increased expression of PC1/3 relative to PC2 in islet alpha cells directs proglucagon processing away from glucagon towards GLP-1 in these situations [16–18]. In contrast, both proconvertase enzymes can process proGIP to biologically active forms GIP(1–42) or GIP(1–30) [19]. This local incretin system within the islets makes a physiological sense given that intestinal-derived incretins are rapidly degraded in the circulation such that their circulating half-lives are less than 10 min and that >95% circulate as inactive GIP(3–42) and GLP-1(9–36) [6,7]. The physiological significance of islet derived incretin peptides has been highlighted also by recent studies in GLP-1 and GIP receptor knock-out mice which indicate the importance of GLP-1 in the expansion of beta cell mass in pregnancy and involvement of both GLP-1 and particularly GIP in islet compensation to cellular stress and hydrocortisone-induced insulin resistance [14,20].

Given that the fat component of the diet is particularly a strong stimulus to GIP release from intestinal K-cells which serves to promote both glucose-induced insulin secretion and fat storage [7], GIP has been proposed to play a prominent role in obesity and underlying derangements of islet cell function and glucose metabolism associated with prolonged consumption of high fat diet. The present work with GIPRKO mice highlights the key role of GIP in islet compensation association with high fat feeding and suggests that defects in GIP action, such as observed in type 2 diabetes, contribute to the process of eventual beta cell demise.

2. Materials and methods

2.1. Animals

Male C57BL/6 mice and GIPRKO mice back-crossed to wild-type C57BL/6 mice for more than ten generations, kindly provided by Professor B Thorens (Lausanne, Switzerland), were bred in house at the Biomedical and Behavioural Research Unit (BBRU) at the University of Ulster, Coleraine [14]. Mice were housed in an air-conditioned room at 22 ± 2 °C with a 12 h light:12 h dark cycle (08:00–20:00 h). Experimental animals had free access to drinking water, and were weaned onto standard rodent diet (10% fat, 30% protein and 60% carbohydrate; energy density of 3.10 kcal/g; Trouw Nutrition, Cheshire, UK) or high fat diet (45% fat, 20% protein and 35% carbohydrate; energy density of 6.13 kcal/g; composition: (g% (w/w): casein, 26.533; choline bitartrate, 0.296; L-cystine, 0.399; lard, 17.895; rice starch, 28.344; cellulose, 6.171; soya oil, 4.319; sucrose, 10.490; mineral mix, 4.319; and vitamin mix, 1.234; Special Diets Service, Essex, UK)), at 3 weeks of age. GIPRKO mice used were confirmed negative for GIPR gene and did not show antihyperglycaemic action of 25 nmol/kg bw GIP when administered together with intraperitoneal glucose (18 mmol/kg) (Fig. 1A–C). All mice were maintained on their respective diets for 45 weeks prior to the collection of blood and tissues together with body fat measurements by DXA scanning (Primus Densitometer, Inside Outside sales, USA). Insulin sensitivity tests (25 U/kg bw) were conducted at the end of the experimental period. All experiments were conducted according to the UK Home Office Regulations (UK Animals Scientific Procedures Act 1986) and the 'Principles of Laboratory Animal Care' (NIH publication No. 86-23, revised 1985).

2.2. Immunohistochemistry

Pancreatic tissues were fixed in 4% paraformaldehyde for 48 h at 4 °C, processed using an automated tissue processor (Leica TP1020, Leica Microsystems, Nussloch, Germany) and then embedded in paraffin wax. Immunohistochemistry was performed as described previously [14,20]. The following primary antibodies were used as appropriate: mouse monoclonal anti-insulin antibody (ab6995, 1:1000; Abcam), guinea-pig anti-glucagon antibody (PCA2/4, 1:200; raised in-house), rabbit anti-GLP-1 antibody (XJIC8, 1:200; raised in-house, specific for total GLP-1), rabbit anti-GIP antibody (RIC34/111J, 1:400; kindly donated by Professor L Morgan, Guildford, UK), and rabbit anti-Ki67 antibody (ab15580, 1:200; Abcam). The following secondary antibodies were used as appropriate: Alexa Fluor 488 goat anti-guinea pig IgG — 1:400, Alexa Fluor 594 goat anti-mouse IgG — 1:400, Alexa Fluor 488 goat anti-rabbit IgG — 1:400 or Alexa Fluor 594 goat anti-rabbit IgG — 1:400. The slides were viewed under a FITC filter (488 nm) or TRITC filter (594 nm) using a fluorescent microscope (Olympus system microscope, model BX51) and photographed using the DP70 camera adapter system. TUNEL assay was used to assess beta cell apoptosis, following manufacturer's instructions (In situ cell death kit, Fluorescein, Roche Diagnostics, UK).

2.3. Image analysis

Islet parameters including islet area, beta cell area and alpha cell area were analysed in a blinded fashion using Cell^F image analysis software (Olympus Soft Imaging Solutions, GmbH) and expressed as μm^2. Islet size distribution (small — islets <10,000 μm^2; medium — >10,000 μm^2 and <25,000 μm^2; large — >25,000 μm^2) was determined using raw data set of total islet area computed using Cell^F image analysis software. Colocalization of glucagon with GIP or GLP-1 was determined using 'Colocalization finder' plugin in ImageJ and expressed as Pearson's colocalization coefficient (−1.0 = no colocalization; 1.0 = full colocalization). Proliferation and apoptosis frequencies were determined by computing percentage of Ki67 and TUNEL positive, insulin positive cells of approximately 2000 beta cells per replicate. The balance between proliferation and apoptosis was expressed as ratio of Ki67/TUNEL.

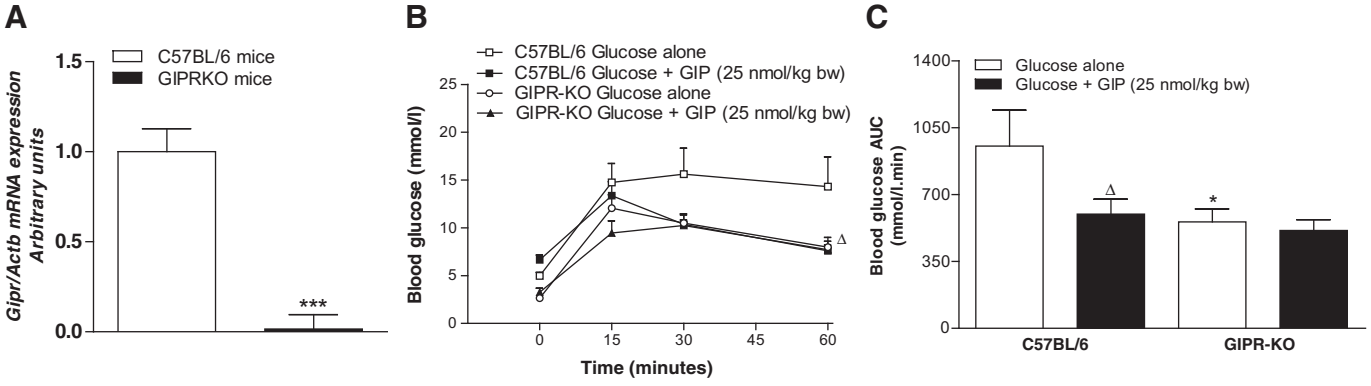

Fig. 1. (A) Expression of *Gipr* mRNA in adipose tissue in C57BL/6 and GIPKO mice. (B) Blood glucose responses of C57BL/6 and GIPRKO mice to glucose alone or glucose with GIP (25 nmol/kg bw). (C) Area under curve. Values are mean ± SEM of 6 observations. $^{*}p < 0.05$, $^{***}p < 0.001$ compared to C57BL/6 mice or glucose alone.

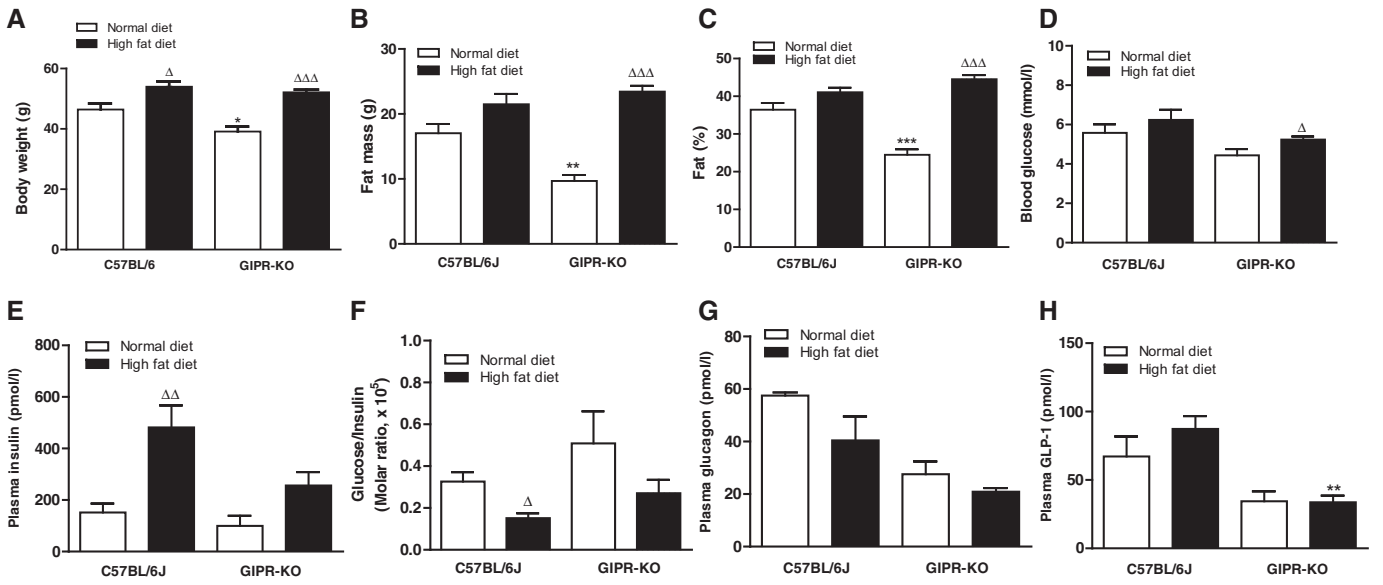

Fig. 2. (A) Body weight, (B) body fat mass (g), (C) body fat (%), (D) blood glucose, (E) plasma insulin, (F) glucose/insulin molar ratio, (G) plasma glucagon, and (H) plasma GLP-1 in C57BL/6J mice and GIPRKO mice fed normal or high fat diet for 45 weeks. Values are mean ± SEM of 6 observations. $^{\Delta}p < 0.05$, $^{\Delta\Delta}p < 0.01$ and $^{\Delta\Delta\Delta}p < 0.001$ compared to similar mice fed normal diet. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to normal mice fed same diet.

2.4. Biochemical analyses

Blood samples were collected in fluoride/heparin microcentrifuge tubes (Sarstedt, Numbrecht, Germany) and centrifuged for 30 s at 13,000 rpm. Plasma was separated and stored at −80 °C until analysis. Plasma glucose was measured by an automated glucose oxidase procedure (Beckman Glucose Analyzer). Pancreatic tissues were extracted using buffer containing 20 mM Tris–HCl (pH 7.5), 150 mM NaCl, 1 mM EDTA, 1 mM EGTA and 1% Triton X 100 and stored at −80 °C. Biochemical analyses were carried out for insulin by radioimmunoassay [21], total GLP-1 (GLP-1 total ELISA, EZGLP-1T-36K, Millipore), GIP (rat/mouse GIP ELISA, EZRMGIP-55K, Millipore) and glucagon (glucagon chemiluminescent assay, EZGLU-30K, Millipore) by specific enzyme linked immunoassays following the manufacturers' instructions. All commercial assay kits have been shown to exhibit a high degree of specificity.

2.5. Statistics

Results were analysed in GraphPad PRISM (Version 5.0) and presented as mean ± SEM. Statistical analyses were carried out by unpaired Student's t test (non-parametric, with two-tailed p values and 95%

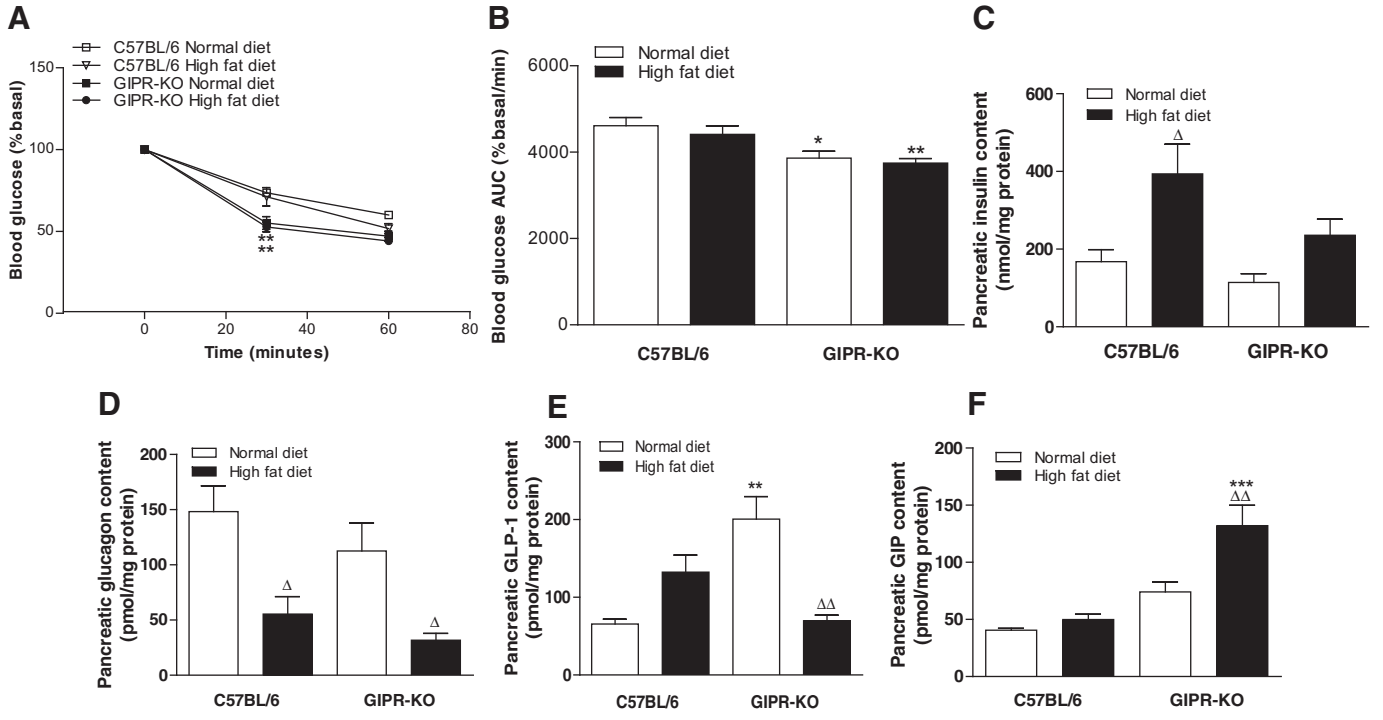

Fig. 3. (A) Insulin sensitivity test — blood glucose as % basal after the administration of insulin (25 U/kg bw). (B) Area under curve and pancreatic contents of (C) insulin, (D) glucagon, (E) GLP-1 or (F) GIP in C57BL/6J mice and GIPRKO mice fed normal or high fat diet for 45 weeks. Values are mean ± SEM of 6 observations. $^{\Delta}p < 0.05$ and $^{\Delta\Delta}p < 0.01$ compared to similar mice fed normal diet. $^{*}p < 0.05$, $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to normal mice fed same diet.

confidence interval) and one way ANOVA with Bonferroni post-hoc test wherever applicable. Results were considered significant if $p < 0.05$.

3. Results

3.1. Glucose homeostasis and body fat

Consumption of high fat diet for 45 weeks did not affect lean body mass but increased body weight, fat mass and fat (%) to the same level in C57BL/6J and GIPRKO mice (Fig. 2A, B, C). This did not cause appreciable change of non-fasting glucose concentrations in C57BL/6J mice but induced insulin resistance as indicated by substantially raised insulin concentrations and decreased glucose/insulin molar ratio (Fig. 2D, E, F). Despite a small increase of circulating glucose levels in HF GIPRKO mice (Fig. 2D), plasma insulin (Fig. 2E), glucose/insulin ratios (Fig. 2F) and glucose concentrations after the administration of exogenous insulin (data not shown) were not significantly changed. When the hypoglycaemic response was expressed as percentage of basal, a small improvement of insulin action was suggested in both normal and high fat fed GIPRKO mice compared with respective C57BL/6 controls (Fig. 3A, B). There were no significant differences in the responses of C57BL/6 and GIPRKO mice to feeding high fat diet (Fig. 3A, B). Plasma glucagon, concentrations tended to be lower in both groups of GIPRKO mice (Fig. 2G), possibly contributing to increased hypoglycaemic action after insulin administration (Fig. 3A, B). Circulating GLP-1 was also decreased, with significantly lower levels observed in GIPRKO mice (Fig. 2H). High fat feeding resulted in marked increases of both fed and 18 h fasted plasma GIP in C57BL/6J mice compared with normal diet controls (218 ± 27 and 98 ± 15 versus 89 ± 24 and 32 ± 2 pmol/l, $n = 4$; $p < 0.05$).

3.2. Islet morphology and hormone contents

Representative images showing insulin and glucagon immunoreactivity in islets of C57BL/6J and GIPRKO mice on normal diet or on high fat diet are shown in Fig. 4A. High fat diet induced significant increase in islet and beta cell areas in all groups of mice (1.8 to 2.5 fold increase, $p < 0.05$, Fig. 4B, C). However, high fat diet induced increase in islet and beta cell areas in GIPRKO mice was significantly less than C57BL/6J mice ($p < 0.001$, Fig. 4B, D). High fat diet also increased alpha cell area in C57BL/6J mice but not in GIPRKO mice (2.5 fold increase, $p < 0.001$, Fig. 4D). The ratio of beta/alpha cell areas was increased by high fat diet but reached significance only in GIPRKO mice (Fig. 4E). Representative images showing the distribution of islets in a random pancreatic section of C57BL/6J and GIPRKO mice on normal diet or on high fat diet are shown in Fig. 5. The number of islets per mm^2 of pancreas was increased in both groups of high fat fed mice (1.5 to 1.8 fold increase, $p < 0.05$, Fig. 5B). However, such an increase in the number of islets in GIPRKO mice was significantly less than high fat fed C57BL/6J mice ($p < 0.05$, Fig. 5B). High fat feeding increased numbers of large sized islets in C57BL/6J mice but not in GIPRKO mice (Fig. 5C).

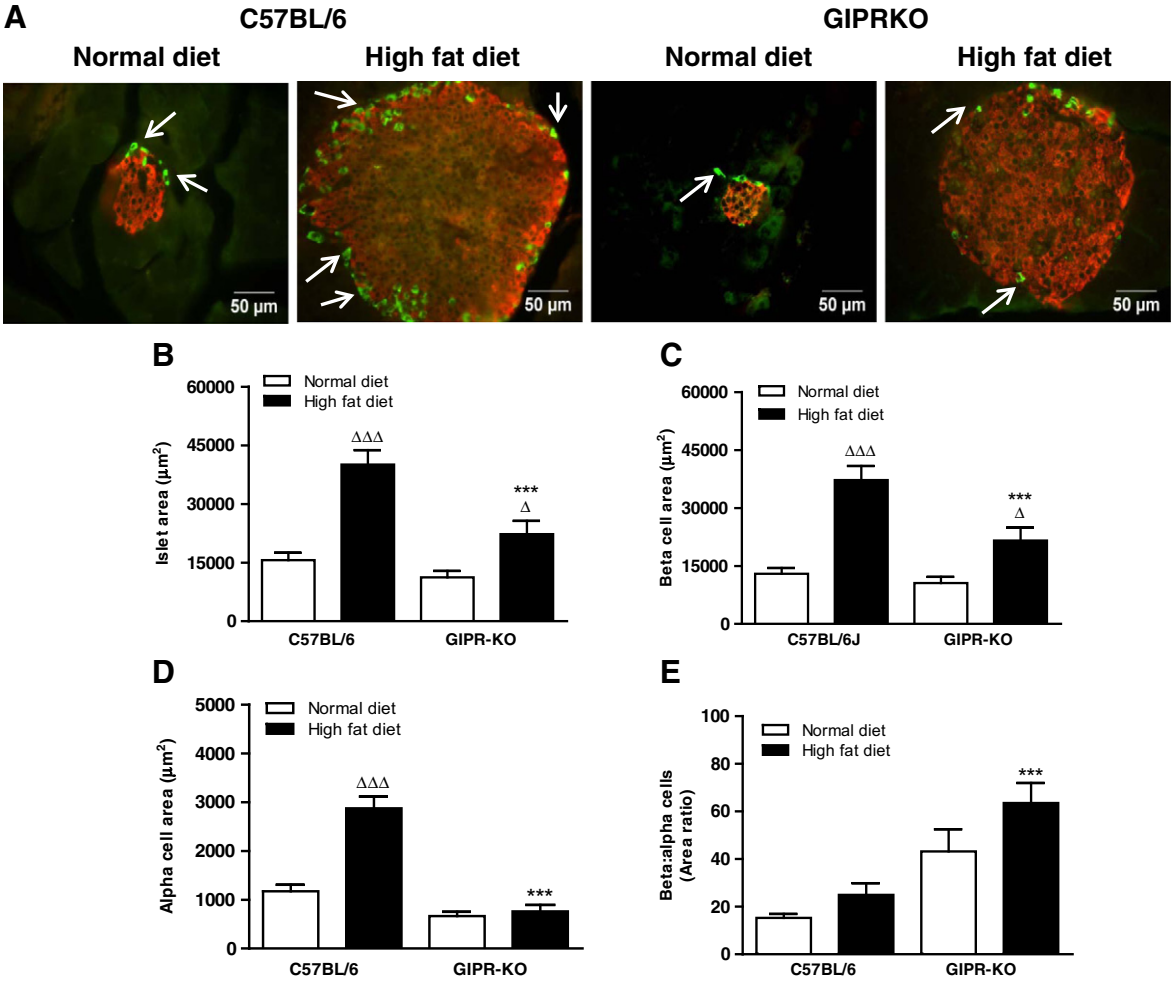

Fig. 4. (A) Representative images of pancreatic islets showing insulin (red) and glucagon (green, indicated by arrows) immunoreactivity from C57BL/6J mice and GIPRKO mice fed normal or high fat diet for 45 weeks. (B) Islet area, expressed as μm^2. (C) Beta cell area, expressed as μm^2. (D) Alpha cell area, expressed as μm^2. (E) Ratio of beta/alpha cell areas. Values are mean ± SEM of 6 observations. $^{\Delta}p < 0.05$ and $^{\Delta\Delta\Delta}p < 0.001$ compared to similar mice fed normal diet. $^{***}p < 0.001$ compared to normal mice fed same diet.

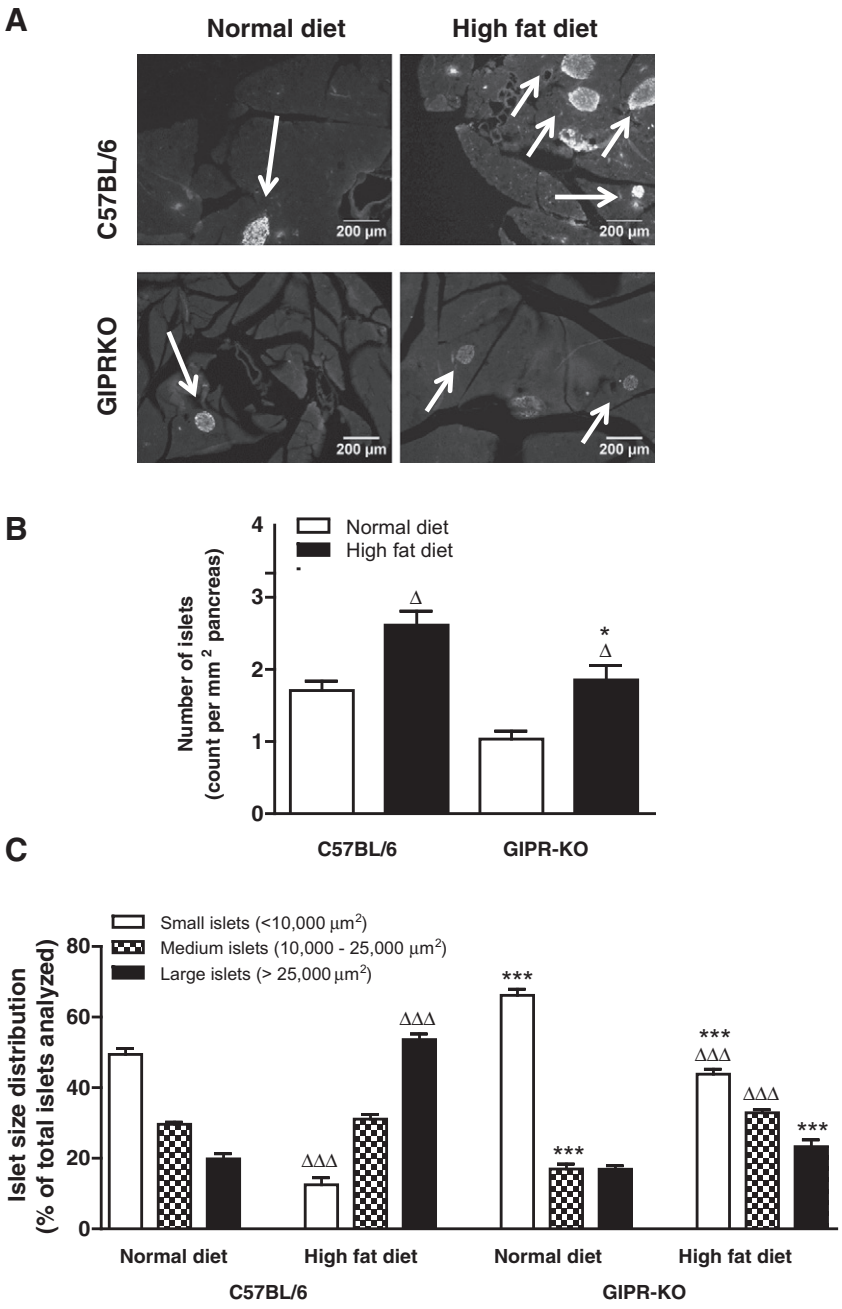

Fig. 5. (A) Representative images of pancreatic islets, (B) numbers of islets per mm^2 of pancreas and (C) islet size distribution in C57BL/6J mice and GIPRKO mice fed normal or high fat diet for 45 weeks. Values are mean ± SEM of 6 observations. $^{\Delta}p < 0.05$ and $^{\Delta\Delta\Delta}p < 0.001$ compared to similar mice fed normal diet. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to normal mice fed same diet.

Representative islets showing Ki67/insulin and TUNEL/insulin staining in islets of C57BL/6J and GIPRKO mice on normal diet or on high fat diet are shown in Fig. 6A. Beta cell proliferation frequency significantly increased in high fat fed C57BL/6J mice (5.3 fold increase, $p < 0.001$, Fig. 6B). A slight increase in beta cell proliferation frequency was observed in high fat fed GIPRKO mice but was less than C57BL/6J mice (1.9 fold but not significant, Fig. 6B). High fat feeding significantly increased beta cell apoptosis frequencies by 2.7 to 2.8 fold in both groups of mice ($p < 0.01$, $p < 0.001$, Fig. 6C). Ki67/TUNEL ratio was markedly higher in high fat fed C57BL/6J mice compared to normal diet ($p < 0.01$, Fig. 6D). No such increase was observed in high fat fed GIPRKO mice.

Consistent with the above changes in beta cell mass, pancreatic insulin content was significantly increased by high fat feeding in C57BL/6J mice but not GIPRKO mice (Fig. 3C). In contrast, pancreatic glucagon content was decreased in both groups of mice (Fig. 3D). Levels of GLP-1 and GIP in the pancreas of C57BL/6J mice were unchanged by high fat feeding, whereas GIPRKO mice exhibited raised levels of pancreatic GIP (Fig. 3E, F). These changes culminated in pancreatic glucagon content being less than GLP-1 and approximately the same as GIP in high fat fed mice (Fig. 3D, E, F).

Representative images showing GLP-1/glucagon and GIP/glucagon immunoreactivity in islets of C57BL/6J and GIPRKO mice on normal diet or high fat diet are shown in Fig. 7. High fat diet did not affect GLP-1/glucagon or GIP/glucagon colocalization in C57BL/6J mice but significantly decreased colocalization in GIPRKO mice ($p < 0.001$, Fig. 7B, C). Such decrease in colocalization coefficient suggests higher expression of GLP-1 or GIP compared to glucagon, evident from GLP-1 or GIP positive

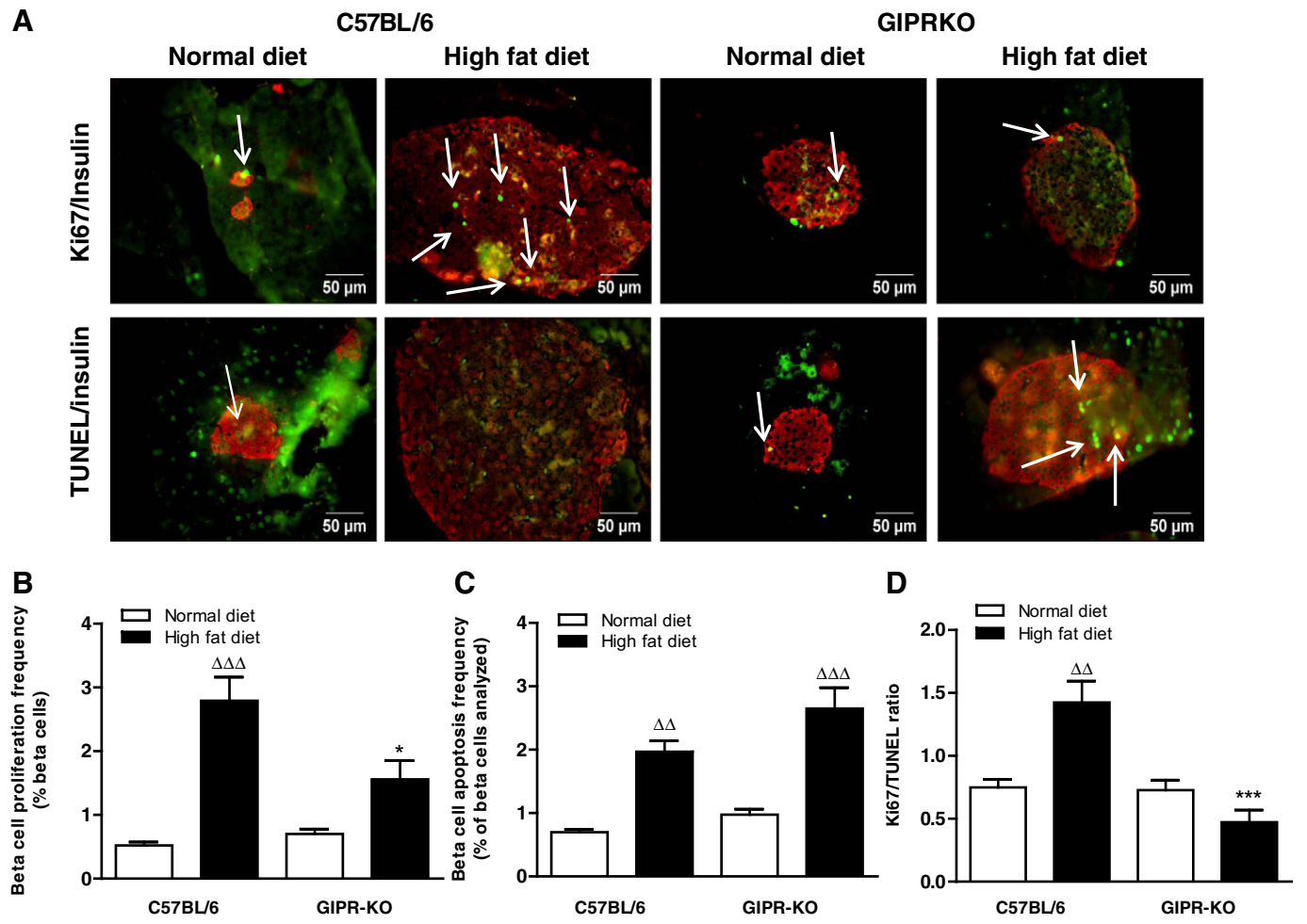

Fig. 6. (A) Representative images of pancreatic islets showing insulin (red) and Ki67 (green, indicated by arrows) or insulin (red) and TUNEL (green, indicated by arrows) immunoreactivity from C57BL/6J mice and GIPRKO mice fed normal or high fat diet for 45 weeks. (B) Beta cell proliferation frequency, expressed as % of beta cells analysed. (C) Beta cell apoptosis frequency, expressed as % of beta cells analysed. (D) Ki67/TUNEL ratio. Values are mean ± SEM of 6 observations. $^{\Delta\Delta}p < 0.01$ and $^{\Delta\Delta\Delta}p < 0.001$ compared to similar mice fed normal diet. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to normal mice fed same diet.

cells with negligible amounts of glucagon, pointed to by broken arrows in Fig. 7A.

4. Discussion

The present study has evaluated islet adaptations to prolonged feeding of high fat diet which was initiated at the time of weaning in C57BL/6 mice. Consistent with previous findings [22], high fat fed mice exhibited increased adiposity as evidenced by measurement of body fat stores by DXA scanning. In contrast to other strains of mice, such as Swiss NIH [23], high fat fed C57BL/6 mice did not exhibit non-fasting hyperglycaemia. Their insulin resistance was modest and compensated by substantial elevation of circulating insulin and small decrease of glucagon, giving rise to a significantly lower circulating glucose/insulin molar ratio compared with mice fed normal laboratory chow. Such strain differences in responses to high fat diets have been noted in mice previously, indicating the important role of genetic factors in metabolic regulation [24].

As expected, the hyperinsulinaemia in high fat fed C57BL/6 mice was driven by a marked elevation of plasma GIP, pancreatic insulin stores, an increase in total islet numbers and a relative increase in the proportion of large (>25,000 μm^2) as opposed to small (<10,000 μm^2) sized islets in the pancreas. Within populations of individual islets, total cellular area was increased, reflecting enhancement of both beta and alpha cell areas. Notably pancreatic stores of glucagon but not GLP-1 were suppressed by high fat feeding. In fact, the major product of proglucagon processing in alpha cells was switched from glucagon to GLP-1, consistent with the activation of PC1/3 under these conditions [17]. Interestingly circulating GLP-1 concentrations were unchanged. This was associated with an increase of both beta cell proliferation and apoptosis but the overall effect was in favour of more than 2-fold increase of beta cell mass. These effects indicate a key role of beta cell replication in the expansion of islet size but further studies are needed to determine whether neogenesis or cellular reprogramming underlies the observed increase of islet numbers [25,26].

It is generally considered that elevated blood glucose due to insulin resistance is an important factor in islet compensation to high fat feeding [27]. However, we have shown recently that insulin resistance induced in mice by hydrocortisone injection provokes significant islet adaptation in the absence of hyperglycaemia [20]. Interestingly, this response was significantly curtailed in mice with knock-out of either GLP-1 or GIP receptors, with strong evidence for key involvement of local islet incretin production in this process. Such observations provide evidence for functional significance of a growing body of data, including those from the present study, indicating significant alpha cell production of both GLP-1 and GIP [15,28,29]. Interestingly in hydrocortisone treated mice, GIP appeared particularly important as numbers of alpha cells mainly producing GIP were markedly increased and animals lacking functional GIP receptors showed a particularly severe loss of islet expansion to hydrocortisone-induced insulin resistance [20].

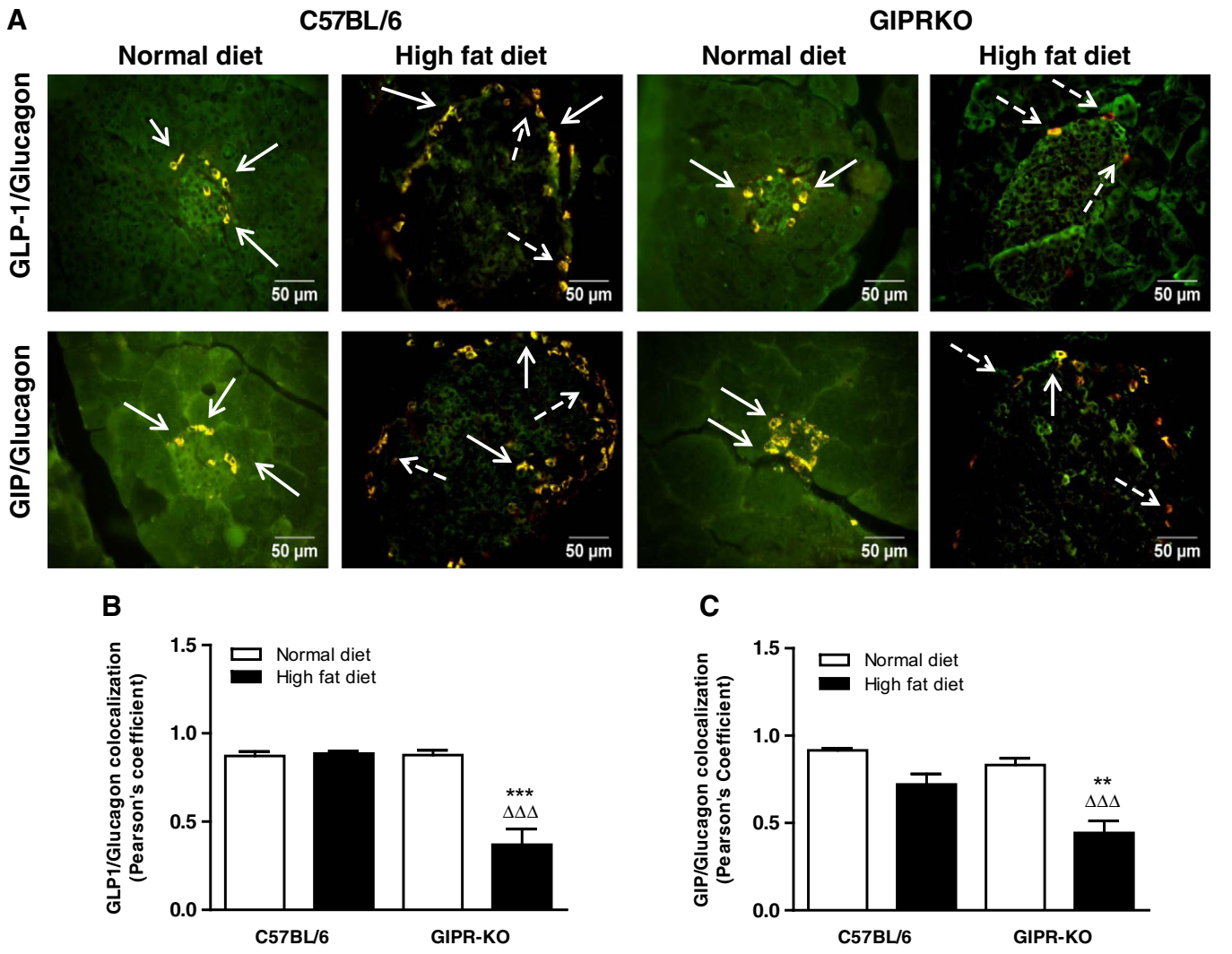

Fig. 7. (A) Representative images of pancreatic islets showing glucagon (green) and GLP-1 (red) or glucagon (green) and GIP (red) immunoreactivity from C57BL/6J mice and GIPRKO mice fed normal or high fat diet for 45 weeks. Solid arrows indicate co-localization of glucagon and GLP-1/GIP while broken arrows indicate alpha cells with negligible amounts of glucagon. (B) GLP-1/glucagon or (C) GIP/glucagon co-localization, expressed in terms of Pearson's coefficient of co-localization. Values are mean ± SEM of 6 observations. $^{\Delta\Delta\Delta}p < 0.001$ compared to similar mice fed normal diet. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to normal mice fed same diet.

To further evaluate the role of GIP in islet biology, the present study has utilised GIPRKO mice [30] to evaluate the possibility of a prominent role of GIP in the compensatory islet response to high fat feeding. These animals exhibited decreased non-fasting glycaemia and a strong trend towards reduced insulin and glucagon levels together with evidence of enhanced insulin sensitivity in hypoglycaemic tests compared to both normal and high fat fed C57BL/6 mice. However, others [31] have shown GIPRKO mice to have unaltered insulin sensitivity and the present study also failed to reveal significant differences in the responses of C57BL/6 and GIPRKO mice to feeding high fat diet. Compared with normal diet controls, GIPRKO mice also exhibited a significant increase of non-fasting blood glucose and enhancement of body fat stores to levels similar to C57BL/6 mice. This is somewhat surprising given that other studies have reported GIPRKO mice to be strongly protected from diet-induced obesity and insulin resistance [32,33]. However, major factors, in addition to differences in the GIPRKO model employed [30,32,33], are likely to concern the duration of the present study which was considerably protracted at 45 weeks. Further, Miyawaki and colleagues [32] reported observations using high fat and control diets, with similar energy densities (3.57 kcal/g). In contrast, the energy density of high fat diet used in the present study was twice as high as control diet (6.13 kcal/g vs 3.10 kcal/g). This tends to suggest that the positive effect of GIPR deletion on insulin resistance [32,34] is less important over time than the negative effect of losing the beneficial actions of GIP on pancreatic beta cells [7,35]. For example, a high fat diet low in carbohydrate may be less detrimental to beta cell function in the longer term than consumption of standard chow rich in carbohydrate as revealed by classical studies in genetically diabetic mice [36].

Further studies are needed to test this hypothesis but the present work has clearly shown that elevation of circulating insulin, increase of pancreatic insulin stores and positive adaptive changes in islet morphology are greatly diminished in GIPRKO mice fed a high fat diet for 45 weeks. This occurs despite similar effect of high fat feeding on insulin sensitivity as observed in C57BL/6 mice. Further, high fat feeding induced a markedly decreased pancreatic GLP-1 content and evoked a non-functional compensatory increase of pancreatic GIP in GIPRKO mice. Thus deletion of GIPR compromised the normal increases of islet number, islet size, beta and alpha cell areas, with abolition of increased beta cell proliferation but a notable enhancement of beta cell apoptosis, giving a significantly decreased cellular Ki67/TUNEL ratio less than unity. Indeed, unlike C57BL/6 high fat fed mice which displayed a Ki67/TUNEL ratio of 1.5, all other groups were undergoing beta cell loss, corresponding to well-known age-related deterioration in the function of insulin secreting cells [37]. Further studies investigating activated caspase 3 levels in beta cells would be useful to assess the extent of apoptosis induction.

These various observations indicate that GIP plays an important role in the normal compensatory islet response to high fat feeding. This likely reflects the loss of the combined actions of intra-islet GIP together with intestinally-derived GIP entering the islet vasculature following secretion from distant K-cells which are stimulated powerfully by dietary fats [7,38]. In this regard, it is notable that the potential action of circulating GIP is limited by extensive DPPIV-mediated degradation which results in a half-life of 7 min, with >95% reaching the islet as inactive GIP(3–42) [39,40]. In contrast, evidence for an effect at islet level concerns a paradoxical increase of pancreatic GIP and remarkable decreases of pancreatic GLP-1 content and GLP-1 staining of the alpha cells in the poorly adapted islets of high fat fed GIPRKO mice. This suggests that part of the beneficial effect of islet derived GIP may be related to positive effects on alpha cell expression and intra-islet actions of GLP-1. Further studies are required to test this hypothesis, including the use of tissue specific GIPRKO mice, but such a relationship between GIP and GLP-1 has been observed in intestinal L-cells [6,38,41]. In addition, it would be of interest to determine whether the decrease in expression of GLP-1 is common to both islet and intestinal-GLP-1.

Overall these data demonstrate that GIP released locally in islets from alpha cells or secreted from intestinal K-cells plays an important role in the positive islet adaptations to high fat feeding. The loss of compensation in GIPRKO mice indicates that the well-known actions of high concentrations of GIP on the stimulation of beta cell proliferation and inhibition of apoptosis [42,43] are physiologically relevant. Such effects are possibly reinforced by positive effects of GIP on the intra-islet production of GLP-1.

Author contributions

Conceived and designed the experiments: PRF. Performed the experiments: SV and RCM. Analysed the data: SV and RCM. Contributed reagents/materials/analysis tools: SV, RCM and PRF. Contributed to the writing of the manuscript: SV, RCM and PRF.

Transparency document

The Transparency document associated with this article can be found, in the online version.

Acknowledgements

This study was funded in part by an EFSD/Boehringer Ingelheim Grant, University of Ulster Research Strategy Funding and a research scholarship to RCM from the Department of Education and Learning, Northern Ireland. Professor B Thorens (Lausanne, Switzerland) is thanked for the provision of GIPRKO mice.

References

[1] V.S. Malik, W.C. Willett, F.B. Hu, Global obesity: trends, risk factors and policy implications, Nat. Rev. Endocrinol. 9 (1) (2013) 13–27.
[2] D.A. Gapp, E.H. Leiter, D.L. Coleman, R.W. Schwizer, Temporal changes in pancreatic islet composition in C57BL/6J-db/db (diabetes) mice, Diabetologia 25 (5) (1983) 439–443.
[3] E.H. Leiter, D.L. Coleman, A.B. Eisenstein, I. Strack, Dietary control of pathogenesis in C57BL/KsJ db/db diabetes mice, Metabolism 30 (6) (1981) 554–562.
[4] J.C. Brown, J.R. Dryburgh, A gastric inhibitory polypeptide II. The complete amino acid sequence, Can. J. Biochem. 49 (1971) 867–872.
[5] J.J. Meier, M.A. Nauck, W.E. Schmidt, B. Gallwitz, Gastric inhibitory polypeptide: the neglected incretin revisited, Regul. Pept. 107 (1–3) (2002) 1–13.
[6] J.J. Holst, The physiology of glucagon-like peptide 1, Physiol. Rev. 87 (4) (2007) 1409–1439.
[7] C.H. McIntosh, S. Widenmaier, S.J. Kim, Glucose-dependent insulinotropic polypeptide (gastric inhibitory polypeptide; GIP), Vitam. Horm. 80 (2009) 409–471.
[8] D.J. Drucker, The biology of incretin hormones, Cell Metab. 3 (3) (2006) 153–165.
[9] C.F. Deacon, J.J. Holst, Dipeptidyl peptidase-4 inhibitors for the treatment of type 2 diabetes: comparison, efficacy and safety, Expert. Opin. Pharmacother. 14 (15) (2013) 2047–2058.
[10] B. Kreymann, G. Williams, M.A. Ghatei, S.R. Bloom, Glucagon-like peptide-1 7–36: a physiological incretin in man, Lancet 2 (8571) (1987) 1300–1304.
[11] M.A. Nauck, Is glucagon-like peptide 1 an incretin hormone? Diabetologia 42 (3) (1999) 373–379.
[12] N. Irwin, V. Gault, P.R. Flatt, Therapeutic potential of the original incretin hormone glucose-dependent insulinotropic polypeptide: diabetes, obesity, osteoporosis and Alzheimer's disease? Expert. Opin. Investig. Drugs 19 (9) (2010) 1039–1048.
[13] M.Y. Donath, R. Burcelin, GLP-1 effects on islets: hormonal, neuronal, or paracrine? Diabetes Care 36 (2) (2013) S145–S148.
[14] 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 (6) (2014) e96863.
[15] Y. Fujita, R.D. Wideman, A. Asadi, G.K. Yang, R. Baker, T. Webber, T. Zhang, R. Wang, Z. Ao, G.L. Warnock, Y.N. Kwok, T.J. Kieffer, Glucose-dependent insulinotropic polypeptide is expressed in pancreatic islet alpha-cells and promotes insulin secretion, Gastroenterology 138 (5) (2010) 1966–1975.
[16] R.D. Wideman, S.D. Covey, G.C. Webb, D.J. Drucker, T.J. Kieffer, A switch from prohormone convertase (PC)-2 to PC1/3 expression in transplanted alpha-cells is accompanied by differential processing of proglucagon and improved glucose homeostasis in mice, Diabetes 56 (11) (2007) 2744–2752.
[17] A.M. Hansen, T.B. Bödvarsdottir, D.N. Nordestgaard, R.S. Heller, C.F. Gotfredsen, K. Maedler, J.J. Fels, J.J. Holst, A.E. Karlsen, Upregulation of alpha cell glucagon-like peptide 1 (GLP-1) in *Psammomys obesus*—an adaptive response to hyperglycaemia? Diabetologia 54 (6) (2011) 1379–1387.
[18] P. Marchetti, R. Lupi, M. Bugliani, C.L. Kirkpatrick, G. Sebastiani, F.A. Grieco, S. Del Guerra, V. D'Aleo, S. Piro, L. Marselli, U. Boggi, F. Filipponi, L. Tinti, L. Salvini, C.B. Wollheim, F. Purrello, F. Dotta, A local glucagon-like peptide 1 (GLP-1) system in human pancreatic islets, Diabetologia 55 (12) (2012) 3262–3272.
[19] R. Ugleholdt, M.L. Poulsen, P.J. Holst, J.C. Irminger, C. Orskov, J. Pedersen, M.M. Rosenkilde, et al., Prohormone convertase 1/3 is essential for processing of the glucose-dependent insulinotropic polypeptide precursor, J. Biol. Chem. 281 (16) (2006) 11050–11057.
[20] S. Vasu, R.C. Moffett, B. Thorens, Flatt, Role of endogenous GLP-1 and GIP in beta cell compensatory responses to insulin resistance and cellular stress, PLoS One 9 (6) (2014) e101005.
[21] P.R. Flatt, C.J. Bailey, Abnormal plasma glucose and insulin responses in heterozygous lean (ob/+) mice, Diabetologia 20 (1981) 573–577.
[22] S.L. Johnston, D.M. Souter, B.J. Tolkamp, I.J. Gordon, A.W. Illius, I. Kyriazakis, J.R. Speakman, Intake compensates for resting metabolic rate variation in female C57BL/6J mice fed high-fat diets, Obesity (Silver Spring) 15 (3) (2007) 600–606.
[23] V.A. Gault, P.L. McClean, R.S. Cassidy, N. Irwin, P.R. Flatt, Chemical gastric inhibitory polypeptide receptor antagonism protects against obesity, insulin resistance, glucose intolerance and associated disturbances in mice fed high-fat and cafeteria diets, Diabetologia 50 (8) (2007) 1752–1762.
[24] S. Andrikopoulos, C.M. Massa, K. Aston-Mourney, A. Funkat, B.C. Fam, R.L. Hull, S.E. Kahn, J. Proietto, Differential effect of inbred mouse strain (C57BL/6, DBA/2, 129T2) on insulin secretory function in response to a high fat diet, J. Endocrinol. 187 (1) (2005) 45–53.
[25] C. Talchai, S. Xuan, H.V. Lin, L. Sussel, D. Accili, Pancreatic β cell dedifferentiation as a mechanism of diabetic β cell failure, Cell 150 (6) (2012) 1223–1234.
[26] M. Van de Casteele, G. Leuckx, Y. Cai, Y. Yuchi, V. Coppens, S. De Groef, N. Van Gassen, L. Baeyens, Y. Heremans, C.V. Wright, H. Heimberg, Partial duct ligation: β-cell proliferation and beyond, Diabetes 63 (8) (2014) 2567–2577.
[27] M.S. Winzell, C. Magnusson, B. Ahrén, Temporal and dietary fat content-dependent islet adaptation to high-fat feeding-induced glucose intolerance in mice, Metabolism 56 (1) (2007) 122–128.
[28] N.M. Whalley, L.E. Pritchard, D.M. Smith, A. White, Processing of proglucagon to GLP-1 in pancreatic alpha cells: is this a paracrine mechanism enabling GLP-1 to act on beta cells? J. Endocrinol. 211 (2011) 99–106.
[29] T.J. O'Malley, G.E. Fava, Y. Zhang, V.A. Fonseca, H. Wu, Progressive change of intra-islet GLP-1 production during diabetes development, Diabetes Metab. Res. Rev. (2014), http://dx.doi.org/10.1002/dmrr.2534 E-pub ahead of print.
[30] F. Preitner, M. Ibberson, I. Franklin, C. Binnert, M. Pende, A. Gjinovci, T. Hansotia, D.J. Drucker, C. Wollheim, R. Burcelin, B. Thorens, Gluco-incretins control insulin secretion at multiple levels as revealed in mice lacking GLP-1 and GIP receptors, J. Clin. Invest. 113 (4) (2004) 635–645.
[31] N. Pamir, F.C. Lynn, A.M. Buchan, J. Ehses, S.A. Hinke, J.A. Pospisilik, K. Miyawaki, Y. Yamada, Y. Seino, C.H. McIntosh, R.A. Pederson, Glucose-dependent insulinotropic polypeptide receptor null mice exhibit compensatory changes in the enteroinsular axis, Am. J. Physiol. Endocrinol. Metab. 284 (5) (2003) E931–E939.
[32] K. Miyawaki, Y. Yamada, N. Ban, Y. Ihara, K. Tsukiyama, H. Zhou, S. Fujimoto, A. Oku, K. Tsuda, S. Toyokuni, H. Hiai, W. Mizunoya, T. Fushiki, J.J. Holst, M. Makino, A. Tashita, Y. Kobara, Y. Tsubamoto, T. Jinnouchi, T. Jomori, Y. Seino, Inhibition of gastric inhibitory polypeptide signaling prevents obesity, Nat. Med. 8 (7) (2002) 738–742.
[33] D. Yabe, Y. Seino, Incretin actions beyond the pancreas: lessons from knockout mice, Curr. Opin. Pharmacol. 13 (2013) 946–953.
[34] V.A. Gault, N. Irwin, B.D. Green, J.T. McCluskey, B. Greer, C.J. Bailey, P. Harriott, F.P. O'Harte, P.R. Flatt, Chemical ablation of gastric inhibitory polypeptide receptor action by daily (Pro3)GIP administration improves glucose tolerance and ameliorates insulin resistance and abnormalities of islet structure in obesity-related diabetes, Diabetes 54 (8) (2005) 2436–2446.
[35] N. Irwin, P.R. Flatt, Evidence for beneficial effects of compromised gastric inhibitory polypeptide action in obesity-related diabetes and possible therapeutic implications, Diabetologia 52 (9) (2009) 1724–1731.
[36] K.P. Hummel, D.L. Coleman, P.W. Lane, The influence of genetic background on expression of mutations at the diabetes locus in the mouse. I. C57BL-KsJ and C57BL-6J strains, Biochem. Genet. 7 (1) (1972) 1–13.

[37] H. Chen, X. Gu, Y. Liu, J. Wang, S.E. Wirt, R. Bottino, H. Schorle, J. Sage, S.K. Kim, PDGF signalling controls age-dependent proliferation in pancreatic β-cells, Nature 478 (7369) (2011) 349–355.
[38] F. Gribble, The gut endocrine system as a coordinator of postprandial nutrient homoeostasis, Proc. Nutr. Soc. 71 (2012) 456–462.
[39] T. Vilsbøll, H. Agersø, T. Lauritsen, et al., The elimination rates of intact GIP as well as its primary metabolite, GIP 3–42, are similar in type 2 diabetic patients and healthy subjects, Regul. Pept. 137 (2006) 168–172.
[40] B. Omar, B. Ahren, Pleiotropic mechanisms for the glucose-lowering action of DPP-4 inhibitors, Diabetes 63 (7) (2014) 2196–2202.
[41] G.E. Lim, P.L. Brubaker, Glucagon-like peptide 1 secretion by the L-cell—the view from within, Diabetes 55 (2) (2006) S70–S77.
[42] A. Trumper, K. Trumper, D. Horsch, Mechanisms of mitogenic and anti-apoptotic signaling by glucose-dependent insulinotropic polypeptide in beta(INS-1)-cells, J. Endocrinol. 174 (2002) 233–2246.
[43] C.H. McIntosh, S. Widenmaier, S.J. Kim, Glucose-dependent insulinotropic polypeptide signaling in pancreatic β-cells and adipocytes, J. Diabet. Investig. 3 (2) (2012) 96–106.