GIP Contributes to Islet Trihormonal Abnormalities in Type 2 Diabetes

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Context: Research and clinical treatments on type 2 diabetes mainly focus on insulin deficiency with little attention paid to other islet hormones.

Objective: This study tested the hypothesis that glucose-dependent insulinotropic polypeptide (GIP) is involved in diabetes-associated multiislet hormone dysregulation.

Design: This paper included a case-control study involving 92 community-based volunteers from the Baltimore Longitudinal Study of Aging (BLSA): 23 with type 2 diabetes on glucose-lowering agents, 25 with newly diagnosed drug-naïve type 2 diabetes, 19 with prediabetes, and 25 with normal glucose tolerance; a separate intervention study with 13 non-BLSA volunteers with type 2 diabetes treated with diet alone, metformin, and/or metformin/sulfonylurea combination; a rodent study; and an in vitro cell line study.

Interventions: An oral glucose tolerance test was performed in the BLSA participants. For the intervention study, saline (0.9% NaCl) or synthetic human GIP (20 ng $\cdot$ kg^{-1} $\cdot$ min^{-1}) was administered to type 2 diabetes subjects for 180 minutes together with a meal, and plasma samples were obtained at predetermined intervals for 360 minutes. A bolus of GIP or placebo was given to C57BL/6 mice.

Main Outcome Measures: Plasma glucose, insulin, glucagon, pancreatic polypeptide (PP), glucagon-like peptide-1 (GLP-1), and GIP were measured.

Results: After an oral glucose tolerance test, glucose, glucagon, PP, GLP-1, and GIP levels were significantly elevated in type 2 diabetes groups, compared with normal and prediabetes groups. GIP infusion in type 2 diabetes subjects was associated with significantly elevated PP levels compared with placebo. The GIP bolus given to C57BL/6 mice was followed by increased PP levels. GIP receptors were found in both human and mouse PP cells.

Conclusions: Up-regulation of GIP production may play an important role in multihormonal dysregulation in type 2 diabetes, most likely through interaction with GIP receptors on islets. (*J Clin Endocrinol Metab* **99**: 2477–2485, 2014)

Research on type 2 diabetes has mainly focused on insulin resistance and insulin deficiency secondary to β-cell failure as causative factors. However, in addition to the effect on β-cells, type 2 diabetes also causes α-cells to secrete excess glucagon and pancreatic polypeptide (PP) cells to produce excess PP. In 1975, Unger and Orci (1) proposed that diabetes was the result of a bihormonal abnormality involving both deficiency of insulin and excess of glucagon levels. Similar to glucagon, plasma PP levels had been shown to be elevated in type 2 diabetes by

Abbreviations: AUC, area under the curve; BLSA, Baltimore Longitudinal Study of Aging; BMI, body mass index; FPG, fasting plasma glucose; GIP, glucose-dependent insulinotropic polypeptide; GIPR, GIP receptor; GLP-1, glucagon-like peptide-1; 2-hrPG, 2-hour plasma glucose; NGT, normal glucose tolerance; OGTT, oral glucose tolerance test; PP, pancreatic polypeptide.

Floyd et al (2) as early as 1976. Although a plethora of studies have examined the causes and consequences of β-cell dysfunction in type 2 diabetes, the causes and consequences of α-cell and PP cell dysregulation remains substantially unknown.

The regulation of glucagon secretion from α-cells is complex and only partially understood. Glucagon secretion is stimulated by hypoglycemia and glucose-dependent insulinotropic polypeptide (GIP) and inhibited by glucagon-like peptide-1 (GLP-1) (3–5). On the contrary, the regulatory mechanisms underlying PP secretion are still unclear. Patients with truncal vagotomy do not exhibit the initial rapid rise in PP levels seen in normal subjects after a meal, suggesting that vagal tone directly or indirectly modulates PP secretion (6). Moreover, the enteroendocrine axis also modulates the typical food-induced PP secretion that occurs after a meal. In fact, iv infusions of glucose, amino acids, or lipids fail to induce changes in plasma PP levels (6).

Several lines of evidence point to incretin hormones such as GLP-1 and GIP as possible candidates for regulating PP secretion: both GLP-1 and GIP are secreted after eating and are secretagogues of at least two islet hormones: insulin via activation of GLP-1 receptors and GIP receptors (GIPRs), and glucagon via GIPRs (4, 7, 8). GLP-1 is not a secretagogue of PP because infusion of GLP-1 or a potent GLP-1 receptor agonist in healthy subjects during hyperinsulinemic-euglycemic and hyperglycemic conditions does not increase PP secretion but rather results in a slight lowering of fasting and postprandial plasma PP levels (6, 9–13). GIP, on the other hand, has been shown to induce PP secretion. Administration of porcine GIP infusion to subjects with or without diabetes increases PP levels 2- to 3-fold (14, 15). In perfused human pancreata, GIP augments PP secretion, which is even further augmented by splanchnic nerve stimulation (16). Therefore, it seems reasonable to conclude that GIP serves as a secretagogue of PP.

In separate studies, different researchers have independently investigated the effects of diabetes on α-, β-, or PP cells or how diabetes affects the actions of GIP on insulin, glucagon, or PP secretion. To date, no study has integrated the association of diabetes with the simultaneous abnormalities of all three islet hormones, and there is no unifying theory underlining GIP as a culprit in these islet hormone abnormalities in diabetes.

We propose that GIP may be one of the major contributors to the hyperglycemia caused by islet trihormonal dysfunction in type 2 diabetes. To test our hypothesis, we present data on plasma levels of glucose, insulin, glucagon, PP, GIP, and GLP-1 during a standard oral glucose tolerance test (OGTT) in four groups of participants charac-terized as having the following: 1) normal glucose homeostasis; 2) impaired glucose tolerance; 3) newly diagnosed diabetes (drug naïve); and 4) diabetes on glucose-lowering medications. We complement our findings with data from a separate GIP infusion study illustrating the effects of exogenous GIP on PP secretion in participants with type 2 diabetes and in C57BL/6 mice. In addition, we show that GIPRs are present on PP-secreting cells in islets of mice and human.

Participants and Methods

BLSA case-control study population

For the case-control study, we selected participants from the Baltimore Longitudinal Study of Aging (BLSA), an ongoing, prospective, observational study of normative aging in community-dwelling volunteers. The BLSA was established in 1958 and has been described in detail elsewhere (17). The BLSA participants, healthy at study entry, undergo about 3 days of medical examination and various testing at the clinical research unit of the National Institute on Aging at predetermined intervals.

A 75-g OGTT was performed in all eligible BLSA participants after a 10-hour overnight fast. Participants on insulin or steroid treatment within 3 months prior to study visit were excluded from OGTT. After consuming a 296-mL solution containing 75 g of glucose, plasma samples were collected at the following 10 time points: baseline (zero minutes, fasting) and 5, 10, 15, 20, 40, 60, 80, 100, and 120 minutes. The fasting plasma glucose (FPG) and 2-hour plasma glucose (2-hrPG) were used to determine the glucose status of each participant using the American Diabetes Association criteria: normal glucose tolerance (NGT) (FPG < 100 mg/dL and 2-hrPG < 140 mg/dL); at risk for diabetes or prediabetes [FPG ≥ 100 mg/dL but < 126 mg/dL (impaired fasting glucose) and/or 2-hrPG ≥ 140 mg/dL but < 200 mg/dL (impaired glucose tolerance)]; or type 2 diabetes (FPG ≥ 126 mg/dL and/or 2-hrPG ≥ 200 mg/dL).

Of the 1362 currently active BLSA participants, we identified 25 participants with newly diagnosed drug-naïve type 2 diabetes (diabetes-new), 23 with type 2 diabetes on glucose-lowering agents (diabetes-med), 19 with prediabetes (only those with both impaired fasting glucose and impaired glucose tolerance were included), and 25 with NGT, matched for age and sex with the diabetes-new group. The characteristics of the study population are shown in Table 1.

Human GIP infusion study

Twenty-two non-BLSA participants with type 2 diabetes treated with diet alone, metformin, and/or a metformin/sulfonylurea combination were recruited to study the effects GIP infusion, compared with placebo, on various hormones, and metabolic parameters after a mixed meal. The results of the main study have been reported previously (4). We had sufficient plasma samples remaining in 13 participants to measure plasma PP levels. These participants had the following characteristics (means ± SD): 10 females, 3 males; age 50 ± 9 years; body mass index (BMI) 38.1 ± 6.9 kg/m^2; hemoglobin A1c 7.2% ± 1.5%; average number of years since diagnosis of diabetes 3.5 ± 3.6 years; and no known diabetes complications. The subjects

Table 1. Characteristics of the Selected BLSA Cohort

Variables	Normal	Prediabetes	Diabetes-New	Diabetes-Med	*P* Trend
Men/women, n	15/10	9/10	15/10	16/7	.406
Age, y	75.7 ± 2.1	74.3 ± 1.7	76.2 ± 2.2	74.4 ± 1.6	.808
BMI, kg/m²	25.5 ± 0.4	25.1 ± 0.3	27.8 ± 0.7[a,e]	28.5 ± 1.1[b,e]	<.001
Hemoglobin A1c, %	5.7 ± 0.1	5.7 ± 0.1	6.4 ± 0.1[c,f]	7.0 ± 0.2[c,f,i]	<.001
Fasting glucose, mg/dL	88.6 ± 1.1	106.0 ± 1.0[b]	117.1 ± 5.4[c,d]	129.6 ± 5.6[c,f,g]	<.001
Glucose AUC, mg/dL·min	14939 ± 522	20800 ± 435[c]	24803 ± 655[c,f]	28126 ± 1021[c,f,h]	<.001
Islet hormones					
Fasting insulin, μU/L	8.0 ± 0.5	9.4 ± 0.9	11.8 ± 1.5	9.5 ± 2.2	.817
Insulin AUC, μU/L·min	6177 ± 539	6881 ± 950	6715 ± 798	3266 ± 656[b,e,h]	.011
Fasting glucagon, pmol/L	11.4 ± 0.8	11.7 ± 0.7	15.0 ± 1.3[a]	14.3 ± 1.0	.047
Glucagon AUC, pmol/L·min	1343 ± 76	1301 ± 73	1651 ± 102[a,d]	1846 ± 179[b,e]	.001
Fasting PP, pmol/L	28.7 ± 2.8	34.1 ± 5.5	39.2 ± 4.8	68.4 ± 9.8[c,f,h]	<.001
PP AUC, pmol/L·min	4237 ± 386	4540 ± 618	6886 ± 667[b]	8771 ± 1067[c,e]	<.001
Enteroendocrine hormones					
Fasting GLP-1, pmol/L	7.0 ± 0.6	8.8 ± 1.1	8.2 ± 0.9	7.7 ± 0.9	.363
GLP-1 AUC, pmol/L·min	957 ± 58	1200 ± 136	1067 ± 70	1324 ± 186[b]	.011
Fasting GIP, pmol/L	11.7 ± 1.3	11.1 ± 2.0	15.6 ± 2.9	19.7 ± 2.4[a,d]	.012
GIP AUC, pmol/L·min	5162 ± 501	5117 ± 563	6830 ± 675[a,d]	6743 ± 526[a,d]	.020

Data presented as means ± SEM. All regression analyses for biomarkers used normalized variables and adjusted for age, sex, and BMI.

[a] *P* < .05 vs normal.

[b] *P* < .01 vs normal.

[c] *P* < .001 vs normal.

[d] *P* < .005 vs prediabetes.

[e] *P* < .01 vs prediabetes.

[f] *P* < .001 vs diabetes-new.

[g] *P* < .05 vs diabetes-new.

[h] *P* < .01 vs diabetes-new.

[i] *P* < .001 vs diabetes-new.

stopped their hypoglycemic medications for 5 days and fasted for 8 hours overnight before each visit. The morning of the study, two iv lines were inserted: one for blood sampling and one for delivery of saline (0.9% NaCl) or synthetic human GIP (20 ng · kg⁻¹ · min⁻¹) (Clinalfa). At time zero, each participant consumed, within 15 minutes, a standardized mixed meal (440 kcal; 56% carbohydrate, 17% protein, and 27% fat). The saline or GIP infusions were started with the first bite of the meal and maintained for 180 minutes. Blood samples were taken at times −20, −10, and zero minutes before the start of the intervention and then every 5 minutes until 75 minutes; every 15 minutes until 180 minutes; and every 20 minutes until 360 minutes after the intervention. Each participant served as his/her own control and returned for the second visit after about 6–12 weeks.

Both the BLSA and the GIP study were approved by the Intramural Research Program of the National Institute on Aging and the Institutional Review Board of the MedStar Health Research Institute in Baltimore, Maryland. Informed consent was obtained from all the participants.

Sample collection

For both the BLSA and the GIP infusion studies, all blood samples were collected into EDTA-coated tubes (1.5 μg/mL blood) containing aprotinin (Trasylol 40 μg/mL blood; Serological Proteins) and a dipeptidyl peptidase 4 inhibitor (10 μg/mL blood; Millipore). After the collection, each sample was centrifuged at 4°C, and the plasma was then divided into several ali-

quots, which were immediately frozen on dry ice and stored at −80°C until analysis.

Plasma hormone and biochemical assays

We quantified plasma glucose levels using a glucose analyzer (Beckman Instruments). Hemoglobin A1c was measured with an automated DiaSTAT analyzer (Bio-Rad Laboratories). The plasma hormones were measured by ELISA or RIA methods according to the kit manufacturers' instructions: GIP (RIA; Phoenix Pharmaceuticals) for the GIP infusion study; GIP (ELISA; Millipore) for the BLSA; GLP-1 (ELISA, Millipore); glucagon (RIA; Millipore); insulin (ELISA; Mercodia Inc); and PP (RIA; Millipore). The specificities, intraassay, and interassay variation of these assays are summarized in Supplemental Table 1.

Animal and cell lines studies

Fasting blood was obtained from the tail veins of chow-fed C57BL/6 4- to 6-week-old male mice (n = 8). A bolus of GIP (20 ng · kg⁻¹) or placebo was given ip, and blood was obtained 15 minutes later for the assay of plasma insulin (rodent insulin ELISA; Crystal Chem Inc) and glucagon and PP (both by the RIA mentioned above).

Human islets (National Islet Cell Resource Center) were fixed in 4% paraformaldehyde for 1 hour, embedded in optimum cutting temperature compound (Tissue-Tek; Electron Microscopy Sciences), frozen, and stored at −80°C. The tissues were later cut

with cryostat, yielding sections with a thickness of 5–7 μm. Mouse pancreata were fixed in 4% paraformaldehyde overnight, washed in PBS, and stored in 4°C until Paraffin embedding. The pancreata were then cut into sections 5–7 um thick and placed on slides. All slides were subject to antigen-retrieval protocols using 1× citrate buffer (BioGenex Laboratories Inc). After antigen unmasking, the slides were cooled to room temperature, permeabilized in 0.2% Triton X-100/PBS for 10 minutes, and blocked with 5% BSA/PBS. These slides were then incubated at 4°C with specific primary antibodies (Supplemental Table 1) and were followed by secondary antibodies (Invitrogen Corp) along with TOPRO-3 (Invitrogen Corp) for nuclear staining. Slides were viewed using a LSM-410 confocal microscope (Carl Zeiss MicroImaging).

Statistical analysis

Results are reported as means ± SE. The area under the curve (AUC) was calculated using the trapezoidal rule and was used as a proxy of the amount of glucose in the circulation or hormones secreted. For the GIP infusion study, AUCs were also divided into positive AUCs and negative AUCs corresponding to the area above and below baseline or fasting levels (t = 0), respectively. The AUC for each curve, AUC_{ALL} (t = 0–360 min), was further divided into different time periods: AUC_{0-60} (t = 0–60 min), AUC_{60-120} (t = 60–120 min), $AUC_{120-220}$ (t = 120–220 min), and $AUC_{220-360}$ (t = 220–360 minutes) to better quantify the changes in the hormonal responses to placebo vs GIP. For the BLSA case-control study, a linear regression model adjusted for covariates was used for a trend analysis across the four different groups. For the GIP infusion study, a paired t test was used to compare the placebo vs GIP infusion intervention. Statistical calculations were carried out using SPSS Statistics version 17.0 (SPSS Inc) and GraphPad Prism, version 5.0 (GraphPad Software).

Results

BLSA case-control study

Characteristics of the selected BLSA cohort

The characteristics of the four groups from the BLSA cohort defined by glucose status are summarized in Table 1. The proportion of men to women and the mean ages of participants among the four groups (NGT, prediabetes, diabetes-new, and diabetes-med) are not statistically different. The BMIs and hemoglobin A1c levels of the NGT and prediabetes participants are similar and are both significantly lower than the two groups of participants with type 2 diabetes (diabetes-new and diabetes-med). The hemoglobin A1c for the diabetes-med group is also significantly higher than the diabetes-new group (P < .01). As expected, FPG and glucose AUC (proxy of glucose disposal) were progressively higher across the four groups of worsening glucose status (Figure 1A) and statistically different from each other (P trend < .001).

Islet hormones during fasting and after oral glucose ingestion

Fasting insulin levels were not different across groups or between groups. However, the dynamics of insulin secretion, as represented by the insulin AUC, was markedly lower in the diabetes-med group in comparison with the normal, prediabetes, and diabetes-new groups (P trend = .011; P < .01 between groups; Figure 1B). Fasting glucagon levels were higher in the diabetes-new and diabetes-med groups (P trend = .047). Compared with fasting glucagon levels, glucagon AUC levels (proxy of glucagon secretion) demonstrated a significant increase in glucagon secretion in both the diabetes groups with the diabetes-med
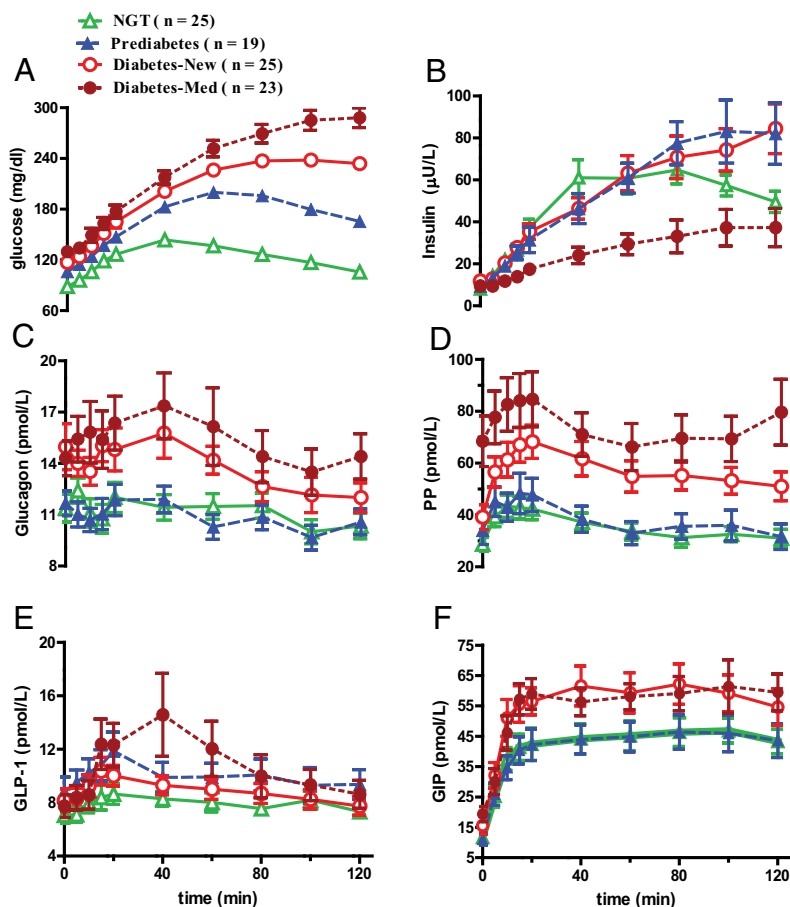

Figure 1. The excursion profile of plasma glucose (A), insulin (B), glucagon (C), PP (D), GLP-1 (E), and GIP (F) after oral glucose ingestion in four groups of selected BLSA cohort (NGT, prediabetes, diabetes-new, and diabetes-med). Data are presented as means ± SE.

group having the highest levels (*P trend* = .001) (Figure 1C). Both fasting PP levels and PP AUC levels showed a trend of progressively higher levels in both the groups with diabetes (*P*

trend < .001), with the levels from the diabetes-med group being significantly higher than those of the normal, prediabetes, and diabetes-new groups (Figure 1D).
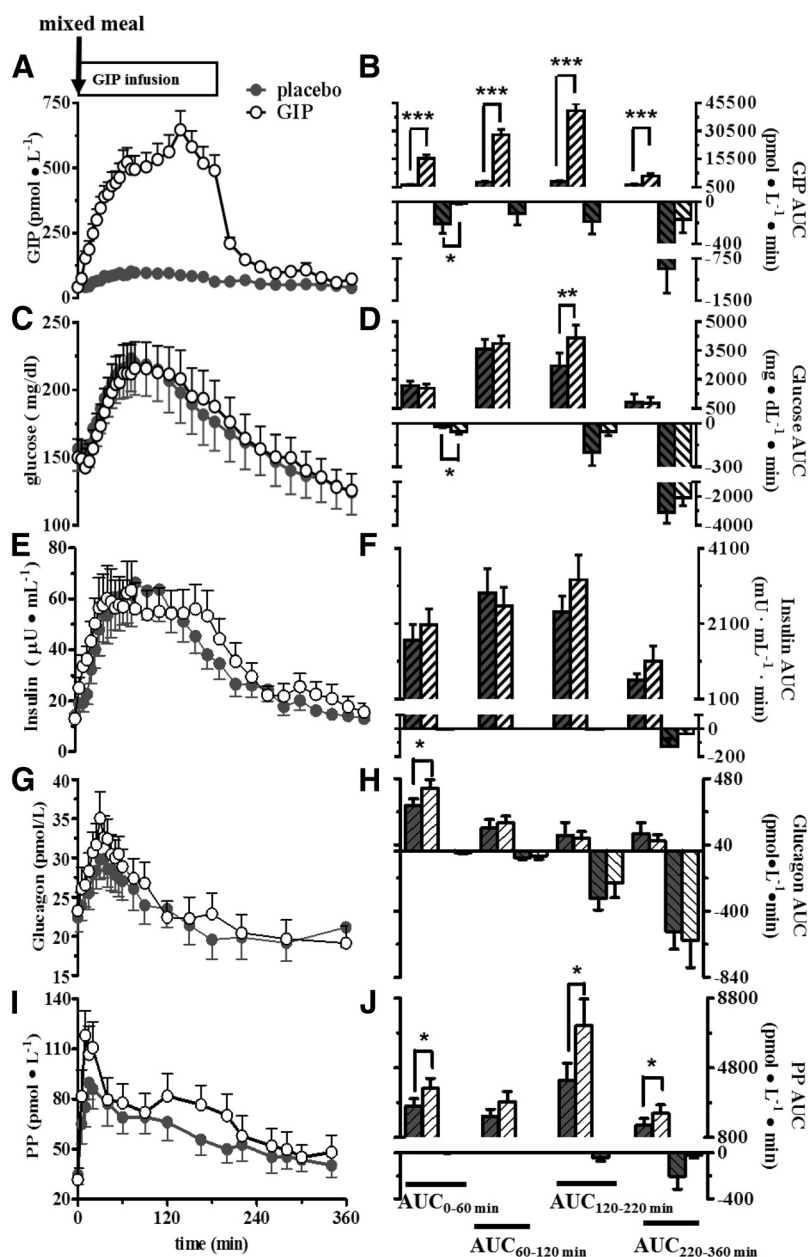

Figure 2. When compared with placebo, exogenous GIP infusion at a pharmacological dose (20 ng·kg^{-1}·min^{-1}) during a mixed meal in 13 participants with type 2 diabetes is associated with the following: a 5-fold significant increase in plasma GIP levels (A); a significant late postprandial increase in plasma glucose levels (120–220 min) (C); no significant difference in insulin levels (E); a significant early postprandial increase in plasma glucagon (0–60 min) (G); and a significant increase in early postprandial (0–60 min) and late postprandial plasma PP levels (120–360 min) (I). GIP or placebo infusion was started a time 0 and continued for 180 minutes. A mixed meal was given at time 0. Data are presented as mean ± SE. Gray close circle, Placebo; black open circle, GIP. The AUC for GIP (B), glucose (D), insulin (F), glucagon (H), and PP (J) during placebo (gray) and GIP infusion (white) is shown. With fasting values (t = 0) serving as baseline levels, positive AUC and negative AUC corresponded to the area above and below the baseline levels, respectively. The AUC for each curve was divided into different time periods: AUC$_{0–60}$ (t = 0–60 min), AUC$_{60–120}$ (t = 60–120 min), AUC$_{120–220}$ (t = 120–220 min), and AUC$_{220–360}$ (t = 220–360 min) to better quantify the changes in the response to the placebo vs GIP infusion. ***, *P* < .001; **, *P* < .01; *, *P* < .05.

Incretin hormones during fasting and after glucose ingestion

There was no difference in fasting GLP-1 levels among the four groups. The only difference found in GLP-1 AUC was between the normal group and the diabetes-med group (*P* < .01) (Figure 1F). As for fasting GIP levels, there was a trend of increasing fasting GIP levels with worsening glucose status (*P trend* = .012) with the diabetes-med group having significantly higher fasting GIP than the normal and prediabetes groups. The diabetes-new and diabetes-med groups also had significantly higher GIP AUC levels when compared with the normal and prediabetes groups (*P trend* =.02; *P* < .05 between groups; Figure 1F).

Human GIP infusion study

As expected, ingestion of a mixed meal resulted in a significant increase over time in plasma glucose, insulin, glucagon, and PP levels in these 13 participants with type 2 diabetes (Figure 2). GIP infusion raised the plasma GIP levels about 5-fold, and significantly enhanced late postprandial elevation of plasma glucose levels AUC$_{120–220}$ (*P* < .01) (Figure 2D) and an early postprandial glucagon level AUC$_{0–60}$ (*P* < .05) (Figure 2H), as we reported previously (4).

GIP infusion was associated with a statistically significant increase in postprandial plasma PP levels in type 2 diabetes

Compared with placebo, GIP administration induced a statistically significant increment in PP secretion during the entire postprandial period AUC$_{0–360min}$ (*P* < .01) in type 2 diabetes. When analyzed separately, PP levels were significantly higher with GIP infusion (*P* = .01) during

early postprandial period AUC_{0-60} and late postprandial period $AUC_{120-360}$, whereas that during AUC_{60-120} was marginally higher ($P = .05$). (Figure 2J).

Animal and cell line studies

To understand how GIP might induce PP secretion, we looked for evidence of the presence of GIPRs in mouse and human islets. Using immunofluorescence, GIPRs were shown to be present in both mouse and human PP cells just as they are present on α- and β-cells (Figure 3, A and B). As previously reported, PP cells are on the rim (or mantle) of mouse islets but are dispersed among the other four cell types in human islets (18). To confirm that GIP modulates PP secretion in another animal model besides humans, we delivered a bolus of GIP (20 ng $\cdot$ kg^{-1}) or placebo to C57BL/6 mice and looked for islet hormones responses. Both plasma glucagon and PP levels but not insulin levels increased significantly at 15 minutes after infusion ($P < .01$, Figure 3, C–E).

Discussion

We have demonstrated that the pathophysiology of type 2 diabetes involves trihormonal abnormalities in islets of Langerhans, specifically involving α-, β-, and PP cells. In this study, we provide evidence from human, animal, and in vitro studies suggesting that the overproduction of GIP in diabetes contributes to the hyperglucagonemia and hyper-PPemia. Data from the BLSA cohort showed that after an oral glucose challenge, GIP secretion is significantly higher in patients with type 2 diabetes than in those with prediabetes or normal glucose metabolism (Figure 1). Administration of GIP during a meal was associated with higher glucagon and PP levels compared with meal alone in participants with type 2 diabetes (Figure 2). GIP-induced glucagon and PP secretion were also demonstrated in rodents (Figure 3). Consistent with a direct regulation of glucagon and PP secretion by GIP, we found GIP-specific receptors on both human and mouse α-cells (4) and PP cells (Figure 3).

In type 2 diabetes, inadequate insulin secretion is accompanied by elevated plasma glucagon levels. In the BLSA cohort, the most noticeable differences between the NGT and the groups with diabetes (diabetes-new and diabetes-med) were the marked elevation in glucagon, PP, and GIP after an OGTT in both diabetes groups (Table 1 and Figure 1). The observed hyperglucagonemia and hypoinsulinemia associated with diabetes are in agreement with the bihormonal abnormality hypothesis of diabetes proposed by Unger and Orci in 1975 (1). A large body of evidence shows that hyperglucagonemia causes increased hepatic glucose output because of increased rates of glycogenolysis and gluconeogenesis from gluconeogenic precursors (19–21). Additionally, glucagon levels, instead of being suppressed, actually increase in type 2 diabetes after oral nutrient intake, which would paradoxically cause even greater glucose output.

The hyper-PPemia observed in diabetes is supported by the findings from Floyd et al (2) in 1976. To our knowledge, however, our study is the first to illustrate the concurrent presence of abnormal function in α-, β-, and PP
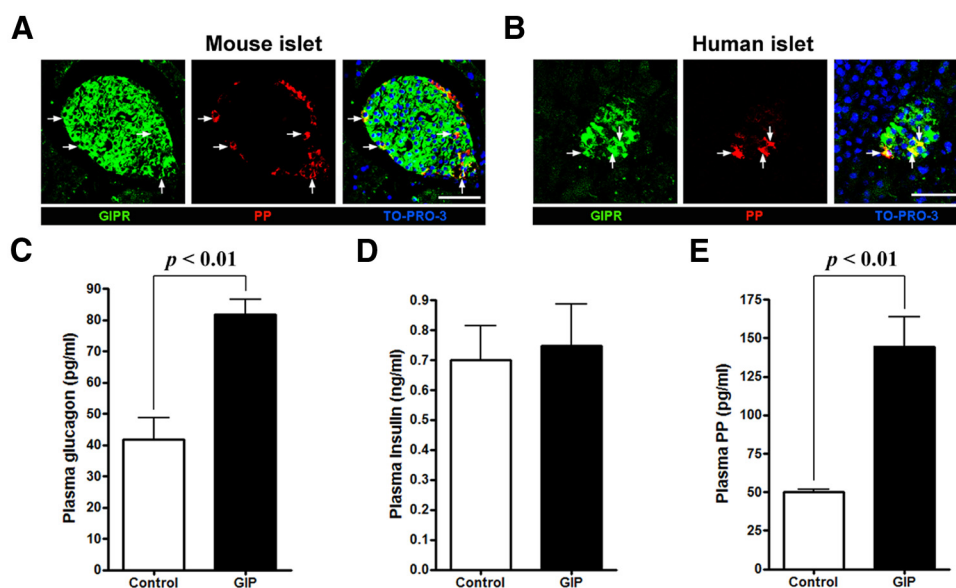

Figure 3. Immunofluorescent images show that GIPRs are colocalized with PP-containing cells in both mouse islet (A) and human islet (B). Green, GIPR; red, PP; blue, nucleus (TO-PRO-3); yellow (with white arrow), colocalization of GIPR with PP. Fifteen minutes after a bolus of GIP was given, C57BL/6 mice had elevated levels of plasma glucagon (82 ± 4.5 pg/mL) (C) and plasma PP (148 ± 8.5 pg/mL) (E) in comparison with saline-injected C57BL/6 mice. White bar, control mice; black bar, mice given GIP bolus. No change in the plasma insulin levels were observed in either group (D).
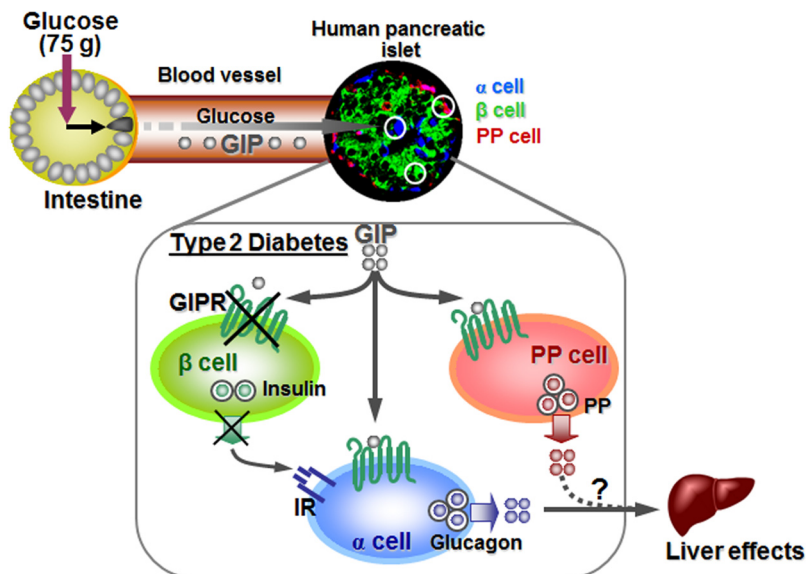
Figure 4. Schema of proposed integrated model representing the islet trihormonal abnormality hypothesis of type 2 diabetes. Upon ingestion of glucose, it is absorbed and simultaneously GIP is released into the circulation from which it interacts with α- (blue), β- (green), and PP cells (red). In type 2 diabetes, GIPRs on β-cells are not responsive to GIP and are therefore ineffective in enhancing insulin secretion response to glucose, even as plasma glucose level gets progressively higher. But GIPRs on α-cells are functional, and their activation causes increased glucagon secretion, unopposed by ineffective insulin secretion. This results in increased hepatic glucose output. GIPRs on PP cells are functional and their activation results in PP secretion. IR, insulin receptor.

gonemia and hypo-PPemia. Intravenous infusion of PP for 8 hours to five patients with chronic pancreatitis improved hepatic insulin resistance (24). Recently Nannipieri et al (25) reported that PP levels were not significant contributors to diabetes remission after Roux-en-Y gastric bypass or sleeve gastrectomy. Unfortunately, GIP levels were not measured in the study. It is possible that the elevated circulating PP levels after a meal are not a contributor to diabetes but a compensatory, although inadequate, response to hepatic insulin resistance and hyperglucagonemia in type 2 diabetes. Therefore, in type 2 diabetes, elevated GIP levels may lead to worsening postprandial hyperglycemia as a result of exacerbated GIP-induced hyperglucagonemia. However, the GIP-induced hyper-PPemia is insufficient to counteract the hyperglycemia, as demonstrated by the GIP infusion study.

We here proposed an integrated physiological model involving a GIP-induced islet trihormonal abnormality hypothesis of type 2 diabetes, an increase in GIP secretion after nutrient ingestion leads to unopposed secretion of glucagon and increased secretion of PP in the setting of diminished insulin secretion (Figure 4). There is evidence that the numbers of L and L/K cells are significantly higher in patients with type 2 diabetes compared with healthy controls (26). Although it has been clearly shown that GIP increases insulin secretion in a glucose-dependent manner, hence its name, the same is not true for glucagon and PP because these hormones responded to a bolus of GIP in the fasting state in mice and humans whereas insulin did not (14, 15). Most likely, within this glucose dependency lies the pathogenesis of GIPR unresponsiveness in type 2 diabetes. Because as glucose levels rise, GIPRs on β-cells become insensitive to activation by GIP (27, 28). However, because GIPRs on PP and α-cells are not glucose dependent for activation, they still respond to circulating GIP levels. The trihormonal abnormalities in type 2 diabetes may also manifest, although not in a similar fashion, in type 1 diabetes. Kennedy et al (29) found that the responses of PP and glucagon to insulin-induced hypoglycemia were impaired or reduced in patients with type 1 diabetes. How the trihormonal hypothesis applies to prediabetes requires further research. Most likely, in-

cells, or trihormonal abnormality, with worsening glycemic control in diabetes. Furthermore, we showed that GIP may be the culprit in the observed hyperglucagonemia and hyper-PPemia in diabetes in the GIP infusion study. Previously we reported that GIP is a secretagogue of glucagon in humans (4) and that GIPRs are present on α-cells in rodent, human islets, and α-cell lines (4, 22). αTC1 cells and purified rat α-cells have increased intracellular cAMP levels and glucagon secretion in response to stimulation with GIP (4, 23). Our data now show that GIP is a secretagogue of PP in humans and mice. This finding is supported by data from perfused human pancreata (16) and human subjects (14, 15). The presence of GIPRs on PP-containing cells in islets in both man and mouse strongly supports the conclusion that the effect of GIP on PP secretion is due to direct signaling. It also appears that PP cells can still respond to GIP in type 2 diabetes because iv GIP infusion during a meal caused a doubling of plasma PP levels above that achieved by meal ingestion alone.

Although the consequences of hyperglucagonemia in type 2 diabetes are well known, the same cannot be said for hyper-PPemia. The role of PP in glucose metabolism remains elusive. There are data pointing to PP being an insulin sensitizer in the liver. Abnormal hepatic glucose metabolism and glucose intolerance are features of chronic pancreatitis. Chronic pancreatitis is also associated with deficient circulating islet hormones such as hypogluca-

sufficient insulin secretion secondary to GIPR down-regulation on β-cells contributes to elevated glucose levels in prediabetes.

Strengths of our studies include the availability and ability to weave together results from a human observation study, a human intervention study, animal models, and in vitro study. However, there are certain limitations. First, the BLSA cohort is older; however, other studies have shown that basal or fasting PP levels were elevated in maturity-onset diabetes compared with a healthy group after adjusting for age (2). Second, the GIP infusion cohort has higher averaged BMI (38 kg/m^2) compared with the BLSA diabetes-med cohort (29 kg/m^2). These differences may explain why the plasma glucagon levels are higher in the GIP infusion group compared with the BLSA diabetes-med cohort, 22 pmol/L vs 14 pmol/L, because higher fasting glucagon levels were observed in obese individuals by other investigators (30).

In summary, type 2 diabetes involves islet trihormonal abnormalities. GIP may play a role in the hyperglucagonemia and the hyper-PPemia seen during the postprandial state in type 2 diabetes when GIPR resistance is present only in β-cells but not in α-cells or PP cells. In type 2 diabetes, hyperglucagonemia exacerbated by elevated GIP levels, may lead to worsening hyperglycemia, which suggests that we should look at GIPR antagonists, not GIPR agonists, as possible treatment options. What role, good or bad, elevated circulating PP levels plays in glucose homeostasis in type 2 diabetes is unclear and warrants further investigation.

Acknowledgments

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