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【 CASE REPORT 】

The Amount of Residual Incretin Regulates the Pancreatic β-cell Function and Glucose Homeostasis

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Abstract:
The gastrointestinal tract is considered an important endocrine organ for controlling glucose homeostasis via the production of incretins. A 21-year-old man emergently underwent total colectomy due to severe ulcerative colitis, and overt diabetes became evident. Weekly administration of a glucagon-like peptide (GLP)-1 receptor agonist (RA) dramatically improved his glucose control. Levels of GLP-1 or gastric inhibitory polypeptide (GIP) were low at the baseline in the duodenum and serum of the patient. After 11 months of GLP-1RA treatment, his HbA1c worsened again, and intensive insulin therapy was necessary to control his glucose levels. Our report may explain the significance of residual incretin for maintaining the pancreatic β-cell function.

Key words: colectomy, diabetes, glucagon-like peptide (GLP)-1, gastric inhibitory polypeptide (GIP)

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Introduction

The gastrointestinal tract is considered an important entero-endocrine organ for controlling whole-body glucose homeostasis by producing glucagon-like peptide (GLP)-1, gastric inhibitory polypeptide (GIP) and other molecules (1, 2). GLP-1 and GIP are major incretin hormones that are released from the gut during meal ingestion and contribute to the incretin effect by potentiating glucose-stimulated insulin secretion and maintain the β-cell mass (1-5). However, the long-term effects of GLP-1 or GIP on pancreatic β-cells in humans have yet to be fully elucidated.

Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by bloody diarrhea and fecal urgency. Since there are no curative treatments, intractable UC can lead to hospitalization and colectomy (6). Patients with total colectomy were previously found to have a hazard ratio of 1.40 for clinically recorded type 2 diabetes (7). However, the exact mechanisms underlying the development of diabetes in patients with colectomy are not fully understood.

We herein report the clinical case of a patient who received total colectomy due to severe UC and developed diabetes with defects in GLP-1 and GIP production. The patient was initially successfully treated with GLP-1 receptor agonist (RA) but subsequently suffered exacerbation of diabetes with a reduction in insulin secretion.

Materials and Methods

Study participants

In addition to the present patient who had diabetes and a history of colectomy (n=1), age-matched non-diabetic, non-colectomy control subjects (young men with a similar body mass index) were recruited for duodenal epithelium sampling (n=3), fecal bacterial 16S rRNA sequencing (n=3) and the measurements of GIP/GLP-1 in serum (n=5).

Written informed consent was obtained from each subject. This study was approved by the institutional review board at Kumamoto University.

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The GLP-1 and GIP expression in the duodenum

Duodenal epithelium samples in the middle part of the descending duodenum from the patient and age-matched non-diabetic, non-colectomy control subjects (n=3) were isolated during an upper-gastrointestinal endoscopic examination. Samples were fixed with 10% natural buffered formalin and then replaced with 30% sucrose for overnight and embedded with optical cutting temperature (OCT) compound (Sakura Finetek Japan, Tokyo, Japan). Frozen thin-sliced duodenal samples (10-μm thickness) from the patient and control subjects were prepared. Incubation with primary antibodies (anti GLP-1 antibody: ab26278, anti GIP antibody: ab22624; abcam, Cambridge, UK) at 1:100 dilution was performed overnight at 4 °C. After washing, the sections were incubated with secondary antibody conjugated with Alexa Fluor 488 or 555 (Molecular Probes, Eugene, USA) for 1 hour at room temperature. Nuclei were stained with DAPI (1:200 dilution) at room temperature for 5 minutes. After rinsing, the sections were mounted with FluorMounting medium (Diagnostic BioSystems, Pleasanton, USA) and examined with a fluorescence microscope (BZ-9000; Keyence, Osaka, Japan).

The measurement of active GLP-1 and GIP

Fasted blood samples were isolated using a BD P800 Blood Collection System (BD, Franklin Lakes, USA). Active GLP-1, GIP and glucagon were measured using corresponding enzyme-linked immunosorbent assay (ELISA) kits [Immuno-Biological Laboratories, Tokyo, Japan; GLP-1: human active GLP-1 (#27784); GIP: human active GIP (#27201); FUJIFILM Wako Pure Chemical (Osaka, Japan)].

Characterization of gut microbiota

Fresh fecal samples were collected after defecation and kept under anaerobic conditions. The DNA of isolated bacteria was extracted using a NucleoSpin®Microbial DNA kit (Macherey-Nagel, Düren, Germany), according to the manufacturer's protocol. The extracted DNA was further purified using an AMPureXP (Bechman Coulter, Pasadena, USA). The 16S rRNA gene was amplified from the DNA extracts using a Bacterial 16S rDNA PCR Kit (Takara Bio, Kusatsu, Japan), and the polymerase chain reaction (PCR) products were purified with AMPureXP. The quality of the sequence library was measured using an Agilent 2200 TapeStation. Clustering and phylogenic classification was performed using QIIME (http://qiime.org/) and an Ribosomal Database Project (RDP) classifier (http://rdp.cme.msu.edu/index.jsp), respectively.

Case Report

A 21-year-old Japanese man started complaining of severe abdominal pain in April 20XX. He underwent total colonoscopy and was diagnosed with UC. His symptoms were severely progressive, so treatment was started with 60 mg prednisolone, 10 mg tacrolimus and 4,000 mg mesalazine. Soon after starting these medications, hyperglycemia of around 300-400 mg/dL with HbA1c 5.2% and glycated albumin (GA) 16.8% became apparent, indicating that he had not previously had diabetes. In May 20XX, emergency subtotal colectomy (from ileo-cecum to sigmoid) was performed due to the acute development of paralytic ileus. After this intervention, his symptoms were resolved, and the medications were appropriately tapered, but his hyperglycemia persisted. Intensive insulin therapy [lispro (6, 6, 6) with glargine biosimilar (0, 0, 0, 8)] was therefore implemented, as shown in Fig. 1. At that time, his body mass index was 18.6 kg/m^2.

Throughout the entire clinical course, his body weight and lifestyle habits were not altered. Anti-glutamic acid decarboxylase (GAD) antibody was <5.0 U/mL, which was confirmed twice and both less than lower limit. Other autoantibodies, such as anti-IA-2 (<0.6 U/mL; cut-off value 0.6) or anti-ZnT8 (<10.0 U/mL; cut-off value 15.0), were negative as well. Urinary C-peptide secretion was 52.3 μg/day. The C-peptide index (CPI) was 0.7, and the ΔC-peptide on a glucagon loading test was 0.4 ng/mL (Table 1). He possessed HLA-DRB1 1503, suggesting that he might be resistant to type 1 diabetes.

In late June, rectumectomy was performed, and total colectomy was completed, so his UC was finally resolved. After the discontinuation of prednisolone and other agents, insulin therapy was switched to treatment with the dipeptidyl peptidase (DPP)-4 inhibitor vildagliptin, but his HbA1c increased from 6.4% to 9.5%. Additional treatment of 1,000 mg metformin and 1 mg glimepiride showed a minor effect on controlling his glucose levels (HbA1c: from 9.5% to 8.2%. Fig. 1).

In consideration of the possibility of defective GLP-1 secretion due to total colectomy, weekly GLP-1 RA with 0.75 mg of dulaglutide was initiated along with the suspension of vildagliptin and glimepiride. After 8 weeks of dulaglutide treatment, his HbA1c dramatically decreased from 8.2% to 5.9%. After 20 weeks of dulaglutide treatment, HbA1c further decreased to 5.4% with no adverse events.

Although GLP-1RA dramatically improved his glucose control, this beneficial effect was not sustained. After 11 months of GLP-1RA treatment, his HbA1c worsened again and increased to 11.3%. Intensive insulin therapy [lispro (12, 10, 10) with glargine biosimilar (0, 0, 0, 12)] was again introduced with GLP-1RA and successfully controlled his HbA1c at 5.3%. At this point, his urinary C-peptide secretion was 26.8 μg/day, his CPI was 0.36, and his ΔC-peptide on glucagon loading test was 0.1 ng/mL (Table 1), indicating that his insulin secretory capacity had decreased to < 50% since his diabetes had been confirmed. The entire clinical course is shown in Fig. 1.

The analysis of GLP-1 and GIP in the duodenum

The first dramatic improvement in glucose homeostasis by GLP-1 RA therapy prompted us to investigate the duodenal GLP-1 and GIP expression, serum active GLP-1 and GIP and compositions of gut microbiota. Duodenal samples were isolated from the patient and age-matched non-diabetic, non-

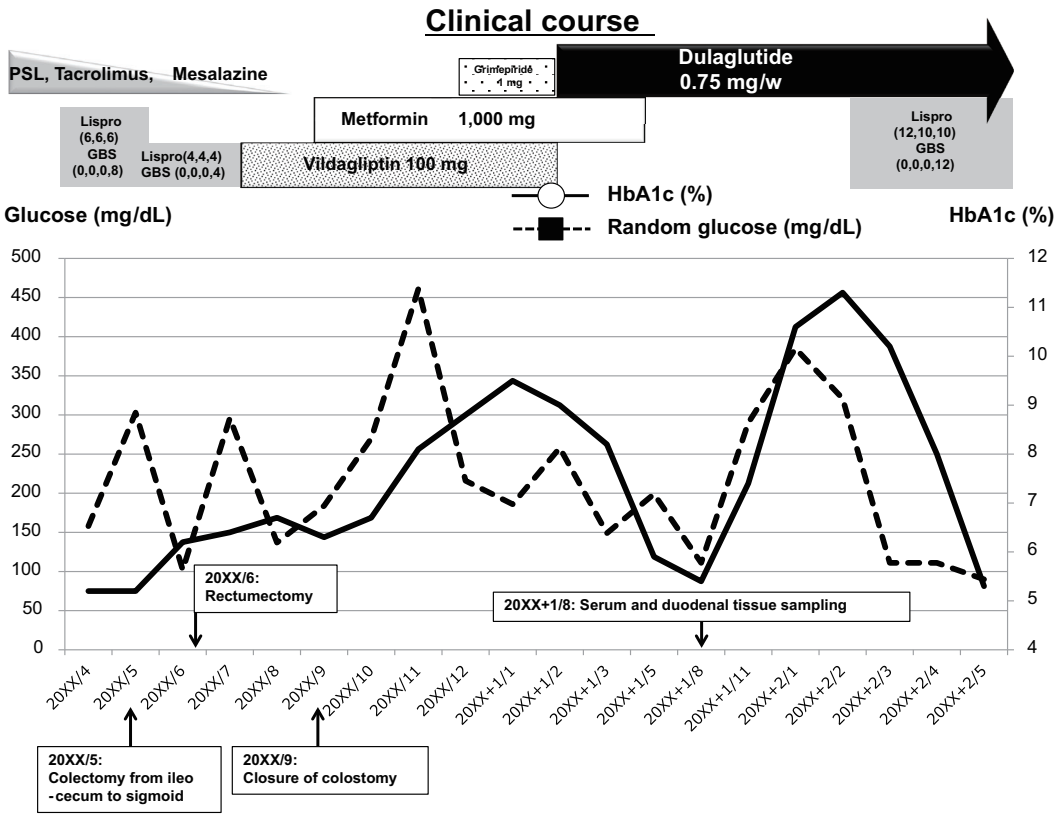

Figure 1. The clinical course of the patient. Medications for the treatment of UC and diabetes, HbA1c (open circle) and fasted glucose (closed square) were indicated. PSL: prednisolone, GBS: glargine biosimilar

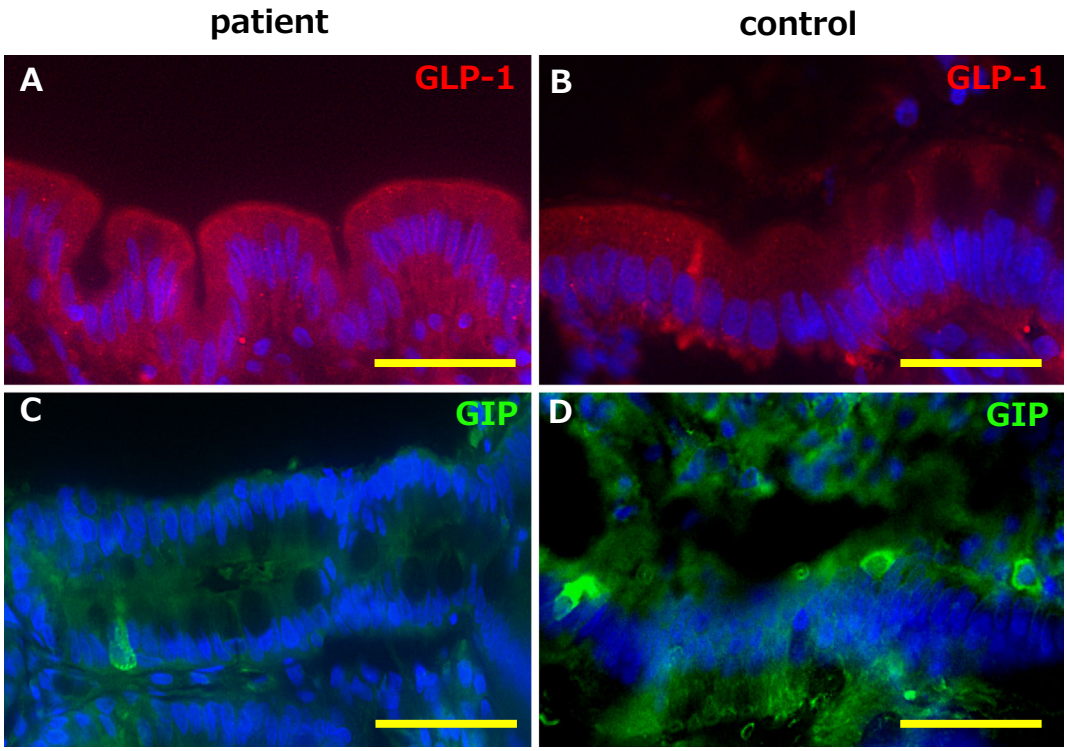

Figure 2. The duodenal expression of GLP-1 and GIP. Frozen sections of duodenal samples from the patient (A, C) and age-matched non-diabetic control subjects (B, D) were fluorescent-immunohistochemically stained for GLP-1 (A, B) or GIP (C, D) with DAPI. The yellow horizontal bar indicates 50 μm in length.

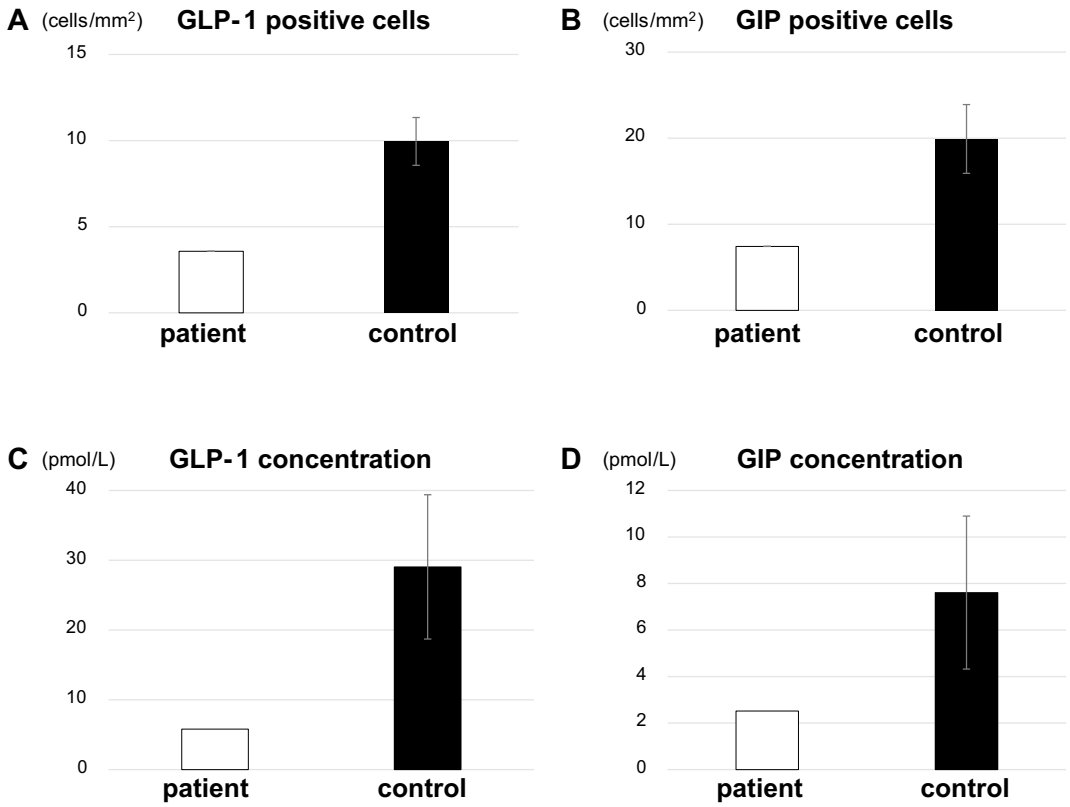

Figure 3. **The density (cells/mm^2) of GLP-1- or GIP-positive cells (A, B). The numbers of GLP-1- or GIP-positive cells (A, B) were quantified using immunohistochemically stained samples. At least 10 different visual fields were independently quantified. Fasted active GLP-1 or GIP concentration (C, D). Fasted blood samples were isolated from the patient and age-matched non-diabetic control subjects using the BD P800 Blood Collection System. Active GLP-1 or GIP (C, D) was measured using corresponding ELISA kits.**

colectomy control subjects (n=3).

The immunohistochemical GLP-1 expression (shown in red; Fig. 2A, B) indicated that GLP-1-positive cells were sparse in the patient but partially positive in the control subjects' duodenum. GIP immunoreactivity (green) in the patient was positive in small populations but more so in the controls (Fig. 2C, D). The quantitative analysis of the density of GLP-1- or GIP-positive cells showed that such cells were rarer in the patient than in the controls (Fig. 3A, B). The fasted serum active GLP-1 level was 5.80 pmol/L in the patient and 29.05±10.34 pmol/L (n=5) in the controls (Fig. 3C), while the GIP level was 2.52 pmol/L in the patient and 7.61±3.29 pmol/L in the controls (Fig. 3D).

The analysis of the gut microbiota

Since the colon possesses a large amount and numerous species of gut microbiota, fecal 16S rRNA sequencing was performed in this patient as well as non-diabetic non-colectomized subjects (n=3). Gut microbiota dysbiosis is responsible for GLP-1RA resistance, and *Lactobacillaceae* numbers were reduced, while *Porphyromonadaceae, Clostridiaceae, Peptostreptococaceae* and *Burkholdericeae* were increased in diabetic mice (8). Our results indicated that the numbers of *Lactobacillales* were comparable among the subjects (Table 4; 0.7% in the patient and 0.2%, 8.2% and 0.2% in controls). The proportion of *Porphyromonadaceae* was 0.0% in the patient and 1.3%, 7.8% and 3.3% in the controls (Table 5). The proportion of *Clostridiaceae* was 16.6% in the patient and 0.9%, 1.4% and 0.1% in the controls (Table 5). The proportion of *Streptococaceae (family)* was 0.5% in the patient and 0.2%, 8.1% and 0.2% in the controls (Table 5). The proportion of *Burkholderiales (order)* was 0.0% in the patient and 4.2%, 0.0% and 1.5% in the controls (Table 4).

Increased numbers of *Firmicutes* and decreased numbers of *Bacteroides* and *Bifidobacterium* are associated with obesity and insulin resistance (9). Among the present study, the proportion of *Firmicutes (phylum)* was 40.8% in the patient and 44.3%, 66.9% and 69.8% in the controls (Table 2). The proportion of *Bacteroidetes (phylum)* was 31.7% in the patient and 41.6%, 31.4% and 20.9% in the controls (Table 2). The proportion of *Bifidobacterium (genus)* was 25.8% in the patient and 2.0%, 0.1% and 3.7% in the controls (Table 6).

Discussion

An increased risk of clinically recorded type 2 diabetes had been reported among patients who underwent total

Table 1. The Changes in Fasted Biochemical Parameters during the Clinical Course of the Patient.

	On admission	After colectomy	GLP-1RA failure	Most recent
	May 20XX	Jan 20XX+1	Feb 20XX+1	Jul 20XX+1
TP (g/dL)	6.4	7.8	7.4	7.5
Alb (g/dL)	2.4	4.9	4.6	4.7
Na (mEq/L)	129	138	134	143
K (mEq/L)	4.9	4.2	4.8	4.3
Cl (mEq/L)	101	102	98	103
Ca (mg/dL)	7.8	9.8	10.1	10.1
IP (mg/dL)	1.7	4.2	4.2	5.2
Mg (mg/dL)	2.4	1.8	1.7	
UA (mg/dL)	1.8	2.7	2.7	3.4
BUN (mg/dL)	10.8	14.4	18.8	18.1
Creatinine (mg/dL)	0.77	0.79	0.75	0.73
eGFR (mL/min/1.73m^2)	>90	>90	>90	>90
Glucose (mg/dL)	177	186	321	111
HbA1c (%)	5.2	9.5	11.3	5.3
GA (%)	16.8	38.6	44.3	16.7
T-Bil (mg/dL)	1.1	2.7	2.2	2.2
AST (U/L)	17	26	19	17
ALT (U/L)	24	14	21	20
LD (U/L)	249	273	289	216
CK (U/L)	45	155	92	80
Fe (μg/dL)	34	101	84	94
UIBC (μg/dL)	202	310	234	273
T-CHO (mg/dL)	128	124	151	163
TG (mg/dL)	56	76	112	57
HDL-C (mg/dL)	63	49	61	72
LDL-C (mg/dL)	60	67	77	86
T-keton (mmol/L)		50.4	136.5	31.2
AcAc (mmol/L)		22.7	65.1	19.6
3-OHBA (mmol/L)		27.7	71.4	11.6
CRP	32.18	0.07	0.33	0.11
GH (ng/mL)		0.4	4.55	
IGF-1 (ng/mL)		233	117	
ACTH (pg/mL)	2.65	31.49	40.91	
cortisol (μg/dL)	0.7	9.5	17.4	
C-peptide (ng/mL)	1.4	1.3		0.4
urine-C-peptide (mg/day)		52.3	26.8	
C-peptide index		0.7		0.36
ΔC-peptide on glucagon load		0.4		0.1
WBC (/μL)	31,900	8,000	8,200	8,900
Baso (%)	0.4	0.4	0.4	0.4
Eosin (%)	0.1	0.5	1.1	0.4
Neut (%)	91.8	70.5	65.6	77.5
Lymph (%)	4.4	23.1	29.6	17.4
Mono (%)	3.3	5.5	3.3	4.3
RBC (×10^6/μL)	4.22	5.81	5.65	5.28
Hb (g/dL)	12.6	15.3	16.6	15.3
Hct (%)	35.2	44.8	46.2	44.6
PLT (×10^6/μL)	517	330	336	321

TP: thyroid peroxidase, Alb: albumin, BUN: blood urea nitrogen, eGFR: estimated glomerular filtration rate, HbA1c: hemoglobin A1c, GA: glycoalbumin, AST: aspartate transaminase, ALT: alanine aminotransferase, CK: creatine kinase, UIBC: unsaturated iron binding capacity, T-CHO: total cholesterol, HDL-C: high-density lipoprotein cholesterol, LDL: low-density lipoprotein cholesterol, AcAc: acetoacetic acid, 3-OHBA: 3-hydorxybutyric acid, CRP: C-reactive protein, IGF-1: insulin-like growth factor-1, ACTH: adrenocorticotropic hormone, WBC: white blood cell, RBC: red blood cell, Hb: hemoglobin, Hct: hematocrit, Plt: platelet

Table 2. Taxonomic Summary: Phylum.

Taxonomy	Patient	Control-1	Control-2	Control-2
k__Bacteria;p__Actinobacteria	25.8%	6.1%	0.4%	7.2%
k__Bacteria;p__Bacteroidetes	31.7%	41.6%	31.4%	20.9%
k__Bacteria;p__Firmicutes	40.8%	44.3%	66.9%	69.8%
k__Bacteria;p__Fusobacteria	0.0%	3.3%	0.4%	0.5%
k__Bacteria;p__Proteobacteria	1.6%	4.7%	1.0%	1.6%

Table 3. Taxonomic Summary: Class.

Taxonomy	Patient	Control-1	Control-2	Control-2
k__Bacteria;p__Actinobacteria;c__Actinobacteria	25.8%	2.0%	0.1%	3.7%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia	0.0%	4.1%	0.3%	3.5%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia	31.7%	41.6%	31.4%	20.9%
k__Bacteria;p__Firmicutes;c__Bacilli	6.3%	0.2%	8.2%	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia	26.3%	43.1%	55.0%	61.2%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi	8.2%	1.1%	3.7%	8.4%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia	0.0%	3.3%	0.4%	0.5%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria	0.0%	4.2%	0.0%	1.5%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria	1.6%	0.6%	1.0%	0.0%

Table 4. Taxonomic Summary: Order.

Taxonomy	Patient	Control-1	Control-2	Control-2
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales	25.8%	2.0%	0.1%	3.7%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales	0.0%	4.1%	0.3%	3.5%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales	31.7%	41.6%	31.4%	20.9%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales	5.6%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales	0.7%	0.2%	8.2%	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales	26.3%	43.1%	55.0%	61.2%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales	8.2%	1.1%	3.7%	8.4%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales	0.0%	3.3%	0.4%	0.5%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales	0.0%	4.2%	0.0%	1.5%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales	1.5%	0.6%	1.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales	0.1%	0.0%	0.0%	0.0%

colectomy, amounting to a 1.4-fold increased incidence (7).

It is widely accepted that GIP-producing K cells are densely populated in the small intestine, while GLP-1-producing L cells are largely distributed in the colon in a reciprocally gradational manner (1, 2, 10). UC patients with colectomy show a slowed release of GLP-1 in response to the intake of glucose (11, 12). The insulin and GIP peak levels are higher in UC patients who have undergone colectomy than non-colectomized controls (11). Thus, glucose homeostasis in colectomized patients with a reduced GLP-1 production may be compensated by an enhanced GIP release.

Given the above, we initially speculated that the present post-colectomy patient might still have certain amounts of incretins in the residual small intestine, but DPP-4 inhibition did not contribute markedly to controlling his glucose levels. Since metformin was suggested to potentially have beneficial effects on the gut microbiota (13), 1,000 mg of metformin was added, but his glucose levels failed to be controlled. A major defect of GLP-1 production was therefore suspected, and weekly GLP-1 RA was initiated, which dramatically improved his glucose levels.

An immuno-histochemical evaluation of the GLP-1- and GIP-producing cells in the residual duodenum revealed that the patient possessed almost no GLP-1-positive cells and had only a few GIP-positive cells compared with control subjects. Consistent with these data, the serum active levels of GLP-1 and GIP in the patient were lower than those in the controls. The defective GLP-1 release in this patient with colectomy may have been caused by the loss of colonic GLP-1-producing entero-endocrine cells. Thus, his incretin production may have been lower at the baseline, with his

Table 5. Taxonomic Summary: Family.

Taxonomy	Patient	Control-1	Control-2	Control-2
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae	25.8%	2.0%	0.1%	3.7%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae	0.0%	4.1%	0.3%	3.5%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae	31.7%	40.3%	22.9%	17.5%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae	0.0%	1.3%	7.8%	3.3%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae	0.0%	0.0%	0.7%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Barnesiellaceae]	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae]	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae	5.6%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae	0.2%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae	0.5%	0.2%	8.1%	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other	0.1%	0.0%	0.0%	0.9%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae	16.6%	0.9%	1.4%	0.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae	0.5%	22.8%	47.0%	43.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae	0.0%	1.5%	3.7%	12.7%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae	9.1%	17.9%	2.9%	4.3%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae]	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae	8.2%	1.1%	3.7%	8.4%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae	0.0%	3.3%	0.4%	0.5%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae	0.0%	4.2%	0.0%	1.5%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae	1.5%	0.6%	1.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae	0.1%	0.0%	0.0%	0.0%

diabetes suddenly triggered by the elimination of his GLP-1 due to total colectomy.

During the clinical course, his glucose control again worsened under single GLP-1RA therapy. Further intensive insulin therapy was thus necessary to control his hyperglycemia, and his insulin-producing capability was found to have been reduced by almost 50% compared with that at the development of his diabetes. Since both his GLP-1 and GIP production appeared to be lower than normal by nature, GLP-1 suspension alone may not have been enough to maintain his pancreatic β-cell mass and/or function. Lower GIP levels may have contributed to a reduction in his β-cell integrity despite the continuous administration of supraphysiological GLP-1. In this context, sudden GLP-1 loss due to colectomy may have immediately caused diabetes (which was able to be restored by GLP-1RA), but long-term GIP loss might gradually further impair his pancreatic β-cell function. Indeed, GLP-1 and GIP double-knockout mice exhibited significantly impaired glucose excursion with decreased insulin secretion (4).

At the onset of diabetes in this patient, his CPI was 0.7 and his ΔC-peptide on the glucagon loading test was 0.4 ng/mL. The efficacy of GLP-1RA monotherapy or sulfonylurea combination depends on the remaining β-cell function (14). A ΔC-peptide on the glucagon loading test of 2.34 ng/mL or CPI of 1.86 have been reported as cut-off values for a longer therapeutic durability of initial GLP-1 RA in Japanese populations (15), suggesting that this particular case already possessed a reduced insulin secretory capacity at the initiation of GLP-1RA. The deterioration of the glucose control with GLP-1RA in the short-term in this patient may have been due to his reduced remaining β-cell function.

Glucose homeostasis in colectomized patients with a reduction in GLP-1 may be compensated for by enhanced GIP release (11). Thus, this compensatory mechanism may not have been sufficient to maintain the β-cell function in this particular case. Indeed, GIP seems to have been quantitatively the most important incretin, particularly with regard to insulin secretion (5). Why this particular patient possessed fewer L- and K-cells by nature is unclear. There was no family history of diabetes, but possible mechanisms underlying the reduced density of these cells include 1) developmental insufficiency, 2) down-regulation and/or 3) dedifferentiation of enteroendocrine cells. Some transcription factors, such as Rfx6, Arx, Pax4 and Isl1, are reportedly important for triggering the differentiation of peptidergic enteroendocrine cells, such as GIP- and GLP-1-secreting cells (16). However, such transcription factors were not evaluated in this patient, so detailed molecular approaches are warranted to clarify the mechanisms underlying the development and/or maintenance of L- and K-cells.

The bacterial load increases along the length of the colon (17), and colectomies involving the left part of the colon are therefore likely lead to the removal of a larger part of

Table 6. Taxonomic Summary: Genus.

Taxonomy	Patient	Control -1	Control -2	Control -2
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Actinomycetales;f__Micrococcaceae;g__Rothia	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Actinobacteria;c__Actinobacteria;o__Bifidobacteriales;f__Bifidobacteriaceae;g__Bifidobacterium	25.8%	2.0%	0.1%	3.7%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Collinsella	0.0%	3.6%	0.0%	3.4%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Eggerthella	0.0%	0.5%	0.3%	0.0%
k__Bacteria;p__Actinobacteria;c__Coriobacteriia;o__Coriobacteriales;f__Coriobacteriaceae;g__Slackia	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Bacteroidaceae;g__Bacteroides	31.7%	40.3%	22.9%	17.5%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Porphyromonadaceae;g__Parabacteroides	0.0%	1.3%	7.8%	3.3%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__Rikenellaceae;g__	0.0%	0.0%	0.7%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Barnesiellaceae];g__	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Butyricimonas	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Bacteroidetes;c__Bacteroidia;o__Bacteroidales;f__[Odoribacteraceae];g__Odoribacter	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Bacillales;f__Bacillaceae;g__Bacillus	5.6%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Carnobacteriaceae;g__Granulicatella	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Enterococcaceae;Other	0.2%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Leuconostocaceae;Other	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Lactococcus	0.0%	0.0%	0.1%	0.0%
k__Bacteria;p__Firmicutes;c__Bacilli;o__Lactobacillales;f__Streptococcaceae;g__Streptococcus	0.5%	0.2%	8.1%	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;Other;Other	0.1%	0.0%	0.0%	0.9%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__;g__	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;Other	1.4%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__Clostridium	6.1%	0.1%	0.0%	0.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Clostridiaceae;g__SMB53	9.1%	0.7%	1.4%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;Other	0.0%	0.2%	1.1%	12.3%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__	0.0%	0.2%	4.2%	7.3%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Anaerostipes	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Blautia	0.0%	4.4%	10.5%	6.5%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Clostridium	0.0%	0.6%	3.9%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Coprococcus	0.5%	1.8%	0.2%	6.6%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Dorea	0.0%	7.1%	4.9%	1.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Lachnospira	0.0%	1.6%	0.0%	0.1%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__Roseburia	0.0%	0.1%	21.1%	3.3%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Lachnospiraceae;g__[Ruminococcus]	0.0%	6.7%	1.0%	6.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;Other	0.0%	0.0%	0.2%	2.9%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Butyricicoccus	0.0%	0.7%	0.4%	0.2%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Faecalibacterium	0.0%	0.1%	0.4%	3.8%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Oscillospira	0.0%	0.7%	2.4%	3.8%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Ruminococcaceae;g__Ruminococcus	0.0%	0.0%	0.4%	2.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Acidaminococcus	0.0%	0.6%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megamonas	0.0%	15.1%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Megasphaera	0.0%	0.0%	0.0%	0.5%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Phascolarctobacterium	0.0%	0.9%	2.9%	3.7%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__Veillonellaceae;g__Veillonella	9.1%	1.2%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Clostridia;o__Clostridiales;f__[Mogibacteriaceae];g__	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;Other	5.7%	0.9%	0.2%	0.0%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__	0.0%	0.0%	0.0%	2.7%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Coprobacillus	0.0%	0.0%	3.3%	0.0%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__Holdemania	0.0%	0.0%	0.2%	0.0%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__[Eubacterium]	2.5%	0.2%	0.0%	5.7%
k__Bacteria;p__Firmicutes;c__Erysipelotrichi;o__Erysipelotrichales;f__Erysipelotrichaceae;g__cc_115	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Fusobacteria;c__Fusobacteriia;o__Fusobacteriales;f__Fusobacteriaceae;Other	0.0%	3.3%	0.4%	0.5%
k__Bacteria;p__Proteobacteria;c__Betaproteobacteria;o__Burkholderiales;f__Alcaligenaceae;g__Sutterella	0.0%	4.2%	0.0%	1.5%
k__Bacteria;p__Proteobacteria;c__Deltaproteobacteria;o__Desulfovibrionales;f__Desulfovibrionaceae;g__Bilophila	0.0%	0.0%	0.0%	0.1%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Escherichia	1.5%	0.6%	0.8%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Enterobacteriales;f__Enterobacteriaceae;g__Klebsiella	0.0%	0.0%	0.1%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Actinobacillus	0.0%	0.0%	0.0%	0.0%
k__Bacteria;p__Proteobacteria;c__Gammaproteobacteria;o__Pasteurellales;f__Pasteurellaceae;g__Haemophilus	0.1%	0.0%	0.0%	0.0%

the colonic microbiota than those involving the right part of the colon (7). A link has been suggested between metabolic diseases and bacterial populations in the gut. Ileum microbiota dysbiosis impairs the GLP-1-induced nitric oxide (NO) production by enteric neurons, which prevents the efficient activation of the gut-brain-to-periphery axis for the control of insulin secretion. Certain peptidoglycans from *Lactobacillaceae* may activate nucleotide-binding and oligomerization domain2/toll-like receptor4/CD14 to produce NO, resulting in enhanced GLP-1 action (8). *Lactobacilli* are positively and *Porphyromonadaceae* negatively correlated with the ileum GLP-1R and neuronal NO synthase mRNA expression (8). In this context, Although GLP-1RA sensitivity may be positively controlled by *Lactobacillaceae* and negatively by *Porphyromonadaceae*, no particular relevance was found in our study.

The absence of microbiota prevented GLP-1-induced insulin secretion, demonstrating a strong GLP-1 resistance (8). The GLP-1R levels were shown to be reduced in a germ-free environment, and β-cells were glucose-unresponsive in the absence of gut microbiota (8). Although the volume of gut microbiota was not examined in the present study, colectomy may have caused reduced numbers of gut microbiota, resulting in GLP-1 resistance. Certain microbiota compositions (i.e. decreased *Firmicutes* and increased *Bacteriodetes* and *Bifidobacteria*) may lead to improved metabolic efficacy. The altered gut microbiota stimulates the differential production of short-chain fatty acids, such as butyrate, that in turn promote GLP-1 secretion from L-cells to improve metabolic health and protect against obesity and diabetes (9). The populations of *Firmicutes* and *Bacteroidetes* were indistinguishable among the subjects (Table 2). The population of *Bifidobacterium* seemed greater in the patient than in the controls (Table 6), but the patient did not receive any metabolic benefits against the development of diabetes. In terms of GLP-1 resistance and GLP-1 production, the composition of gut microbiota in this patient may not have had any major effects on controlling glucose homeostasis.

Since we do not have colectomized non-diabetic controls or more patients with diabetes due to UC colectomy, the analysis of the gut microbiota is just observational but not conclusive. As the residual small intestine takes over the function and microenvironment of the resected colon, our observation in gut microbiota may not be directly related to the diabetic phenotype in this patient but instead represent a transition in colonization of the residual small intestine. Thus, changes in the gut microbiota may not have been involved in the development of diabetes in this case.

We encountered a clinical case of a patient who underwent total colectomy due to severe UC and developed diabetes with defects in GLP-1 and GIP production. He was initially successfully treated with GLP-1 RA. The later worsening of glucose control, represented by a reduced insulin secretory capacity, suggests the importance of GIP for the long-term maintenance of pancreatic β-cells.

The development of diabetes in this particular patient may have been due to the elimination of colon GLP-1-producing cells by colectomy, based on the spontaneously reduction in his GLP-1/GIP production capability. The short-term effectiveness of GLP-1RA treatment suggested that prolonged GIP loss might cause gradual pancreatic β-cell dysfunction. Our report may explain the importance of residual incretins for maintaining the pancreatic β-cell function. This case report provides additional insight into the spatiotemporal role of incretins of the intestinal system in the control of whole-body glucose homeostasis.

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