OBES SURG (2014) 24:2075–2081
DOI 10.1007/s11695-014-1279-x

ORIGINAL CONTRIBUTIONS

Obesity Associated with Type 2 Diabetes Mellitus Is Linked to Decreased PC1/3 mRNA Expression in the Jejunum

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Published online: 16 May 2014
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Abstract
Background Bariatric surgery is the most effective therapeutic option for obesity and its complications, especially in type 2 diabetes. The aim of this study was to investigate the messenger RNA (mRNA) gene expression of proglucagon, glucose-dependent insulinotropic peptide (GIP), prohormone convertase 1/3 (PC1/3), and dipeptidyl peptidase-IV (DPP-IV) in jejunum cells of the morbidly obese (OB) non type 2 diabetes mellitus (NDM2) and type 2 diabetes mellitus (T2DM), to determine the molecular basis of incretin secretion after bariatric surgery.
Methods Samples of jejunal mucosa were obtained from 20 NDM2 patients: removal of a section of the jejunum about 60 cm distal to the ligament of Treitz and 18 T2DM patients: removal of a section of the jejunum about 100 cm distal to the ligament of Treitz. Total RNA was extracted using TRIzol. Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) was carried out. Samples were sequenced to PC1/3 by ACTGene Análises Moleculares Ltd. Immuno content was quantified with a fluorescence microscope.
Results T2DM showed decreased PC1/3 mRNA expression in the primers tested (primer a, $p=0.014$; primer b, $p=0.048$). Many patients (36.5 %) did not express PC1/3 mRNA. NDM2 and T2DM subjects showed nonsignificantly different proglucagon, GIP, and DPP-IV mRNA expression. The immuno contents of glucagon-like peptide-1 and GIP decreased in T2DM jejunum, but incubation with high glucose stimulated the immuno contents.
Conclusions The results suggest that bioactivation of pro-GIP and proglucagon could be impaired by the lower expression of PC1/3 mRNA in jejunum cells of obese patients with T2DM. However, after surgery, food could activate this system and improve glucose levels in these patients.

Keywords Glucagon-like peptide-1 · Glucose-dependent insulinotropic peptide · Dipeptidyl peptidase-IV · Prohormone convertase · Type 2 diabetes mellitus · Morbidly obese

Abbreviations

GIP	Glucose-dependent insulinotropic peptide
GLP-1	Glucagon-like peptide-1
PC1/3	Prohormone convertase 1/3
DPP-IV	Dipeptidyl peptidase-IV
mRNA	Messenger RNA
RT-qPCR	Reverse transcription quantitative real-time polymerase chain reaction
T2DM	Type 2 diabetes mellitus
NDM2	Non type 2 diabetes mellitus
OB	Obese
NOB	Nonobese

Introduction

Obesity is becoming a worldwide epidemic. It is a major risk factor for cardiovascular disease and is directly linked to the development of type 2 diabetes (World Health Organization Global Infobase, www.who.int/mediacenter). Bariatric surgery is the most effective therapeutic option for reduction

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of weight and its related complications in individuals with severe obesity (BMI≥40 kg/m^2) [1–4]. Postoperative changes in gastrointestinal anatomy and function help normalize the metabolic syndrome parameters [5–9], owing to the removal of part of the gastrointestinal tract which has important endocrine activity [10]. These endocrine cells produce, among other substances, glucose-dependent insulinotropic peptide (GIP), secreted in the duodenum and proximal jejunum by K cells, and glucagon-like peptide-1 (GLP-1), synthesized and released by L cells from the medium jejunum to rectum [4]. These are key modulators of insulin secretion, glucose homeostasis, and gastric emptying [11–13].

The precursors of GIP and GLP-1 are cleaved by the enzyme prohormone convertase 1/3 (PC1/3), which is the intestinal K and L-cells, respectively [14, 15]. The action of incretins is completed by the cleavage of N-terminal peptides by a tissue and serum dipeptidyl peptidase-IV (DPP-IV) [16, 17].

GLP-1 release was found to be lower in obese (OB) compared to nonobese (NOB) subjects [18]. Studies have demonstrated that GIP secretion remains unchanged, but plasma concentrations of GLP-1 decreased in type 2 diabetes mellitus (T2DM) subjects [19, 20]. GLP1 secretion decreased in obese individuals with T2DM, affecting the incretin signaling process on satiety. GLP-1 promotes retardation of gastric emptying by stimulating insulin release, inhibition of gastric acids, thereby regulating the passage of nutrients through the gastrointestinal tract [6]. Other studies showed that serum levels of GLP-1 increased considerably in obese T2DM and obese non type 2 diabetes mellitus (NDM2) patients after bariatric surgery [3, 10, 21], and clinical data showed improved glycemic control in these individuals [1, 8]. It is possible that food intake after surgery directly stimulates the L-cells in the medium jejunum to release GLP-1 [5, 6, 22].

The aim of this work was to investigate the basal pattern of messenger RNA (mRNA) expression of proglucagon, GIP, PC1/3, and DPP-IV in jejunum cells of obese patients with NDM2 and T2DM. So, mRNA levels of these genes of interest were assessed to determine the molecular basis of incretin secretion intended for the metabolic alterations in morbidly obese subjects who underwent bariatric surgery.

Materials and Methods

Samples

Proximal jejunum mucosal samples were obtained from 20 OB NDM2 patients (control group) and 18 OB T2DM patients, who underwent bariatric surgery in COM/PUCRS, Brazil. A section of the jejunum about 60 cm distal to the ligament of Treitz was removed from OB NDM2 patients and a section about 100 cm from T2DM OB patients. Proximal jejunum mucosal samples of nine NOB and NDM2 patients (eutrophic control) were collected 30 cm distal to the ligament of Treitz, as in a routine endoscopic investigation. Experiments were approved by the ethics committee of the Federal University of Rio Grande do Sul (no. 2007949), and all subjects were informed on the aim of the study and signed the informed consent form.

Analysis of Human Gene Expression

The collected samples were immediately immersed in TRIzol reagent (Invitrogen, Darmstadt, Germany). Approximately 2 μg of total RNA was added to each cDNA synthesis reaction using the SuperScript II-TEN preamplication system (Invitrogen). Reactions were performed at 42 °C for 1 h using the first T23V (5′TTT TTT TTT TTT TTT TTT TTT TTV). Reverse transcription quantitative real-time polymerase chain reaction (RT-qPCR) amplification was carried out using specific primer pairs for proglucagon (precursor of GLP-1), GIP, DPP-IV, prohormone convertase (PC1/3) primers "a" and "b," and the internal control β-actin. We reported that the primers of PC1/3, selected by GenBank (http://wwww.ncbi.nlm.nih.gov) did not detect the mRNA of PC1/3 in all patients. We tested two primers (indicated a and b) that were able to anneal and amplify the cDNA produced in some patients (Table 1). RT-qPCRs were carried out in an Applied Biosystem-7500 real-time cycler. Reaction settings comprised an initial denaturation step of 5 min at 95 °C, followed by 40 cycles of 15 s at 95 °C, 10 s at 60 °C, 15 s at 72 °C, and 35 s at 60 °C; samples were held for 15 s at 95 °C for annealing and then heated for 1 min to 60 °C with a ramp of 0.1 °C/s to acquire data to produce the denaturing curve of the amplified products. RT-qPCRs were carried out in a final volume of 20 μl, composed of 10 μl reverse transcript of each sample diluted 10 to 100 times, 2 μl of 10× PCR buffer, 1.2 μl of 50 mM $MgCl_2$, 0.1 μl of 5 mM dNTPs, 0.4 μl of 10 μM primer pairs, 4.25 μl of water, 2.0 μl of SYBR Green (1:10,000 Molecular Probe), and 0.05 μl of Platinum Taq DNA Polymerase (5 U/μl) (Invitrogen).

Gene Sequencing of PC1/3

Samples were sequenced by ACTGene Análises Moleculares Ltd. (*Centro de Biotecnologia*, UFRGS, Porto Alegre, Brazil) using the automatic sequencer ABI Prism 3100 Genetic Analyzer armed with 50-cm capillary sets and POP6 polymer (Applied Biosystems). DNA templates (60 ng) were labeled with 2.5 pmol of the primer (PC1/3, Table 1) and 3 ml of BigDye Terminator v3.1 Cycle Sequencing RR-100 (Applied Biosystems) in a final volume of 10 ml. Labeling reactions were performed in a GeneAmp PCR System 9700 thermocycler (Applied Biosystems) with an initial denaturing step of 96 °C for 3 min followed by 25 cycles of 96 °C for

Table 1 Oligonucleotides used in RT-qPCR reactions, 5′ to 3′

Gene	Forward primer	Reverse primer
β-Actin	5′ CCACGAAACTACCTTCAACTCC 3′	5′ TCATACTCCTGCTGCTGCTTGCTGATCC 3′
PC1/3 a	5′ GACCTCTTCTCAGCCAGAGC 3′	5′ GACACCAGGGTGTTCTCCTT 3′
PC1/3 b	5′AGAGGGGTGGAGAAGATGGT3′	5′ GCTTTGGCGGTGAGTTTTTA 3′
GIP	5′ AACCCCAGCGATGAAGATTT 3′	5′ TGAGGCAGGTGCTAAGTGAA 3′
Proglucagon	5′ GCCAGGGACTTTATAAACTGGT 3′	5′ AAGCAATGTGGCCTCAGAAT 3′
DPP-IV	5′ TCCTTATTCATGGAACAGCAGA 3′	5′ TGAAGTGGCTCATGTGGGTA 3′

10 s, 55 °C for 5 s, and 60 °C for 4 min. Labeled samples were purified by 75 % isopropanol precipitation followed by 60 % ethanol rinsing. Precipitated products were suspended in 10 ml Hi-Di formamide (Applied Biosystems), denatured at 95 °C for 5 min, ice-cooled for 5 min, and electroinjected in the automatic sequencer. Sequencing data were collected using Data Collection v 1.0.1 software (Applied Biosystems) designed with the following parameters: Dye Set "Z," Mobility File "DT3100POP6{BDv3}v1.mob," BioLIMS Project "3100_Project1," Run Module 1 "StdSeq50_POP6_50cm_cfv_100," and Analysis Module 1 "BC-3100SR_Seq_FASTA.saz."

Incubation with High Glucose

Samples were collected during surgery and were immediately immersed in ice-cold Hanks' Balanced Salt Solution (HBSS) with 0.65 mM dithiothreitol (DCT, Sigma), 1 % bovine serum albumin (BSA; Sigma Chemical Co., St. Louis, MO), 10 ml/l penicillin/streptomycin (Sigma), 9.05 mM $NaHCO_3$ (Synthi), and 20 mM HEPES (Sigma) [23]. Sliced sample (400 μm) produced in chopper, was washed in a 12-well culture plate containing 1 ml of the same solution per well. After removing the HBSS, 1 ml of DMEM (Gibco) was added supplemented with 10 ml/l penicillin/streptomycin, 1 mg/ml hydrocortisone (Sigma), 4 ng/ml insulin (Sigma), 5 ng/ml nerve growth factor (Sigma), and 0.1 % BSA (Sigma Chemical Co., St. Louis, MO) [23] without (control) or with 11 mM glucose. Slices were incubated for 2 h at 37 °C in 5 % CO_2. Each treatment was incubated in triplicate. After incubation, the medium was removed and 300 μl of TRIzol was added for RNA extraction.

Gene Expression Analysis

We quantified gene expression using the $2^{-\Delta\Delta Ct}$ (threshold cycle) method [24]. For each sample, analyzed in quadruplicate, a ΔCt value was obtained by subtracting the β-actin Ct value from the Ct of the gene of interest. OB NDM2 (control group) patients were used as the calibrator group. All genes were analyzed taking this patient group as a reference, to calculate the relative ΔΔCt for each gene.

Immunohistochemistry

Samples were collected during surgery and were immediately immersed in ice-cold HBSS with 0.65 mM dithiothreitol (DCT, Sigma), 1 % BSA (Sigma Chemical Co., St. Louis, MO), 10 ml/l penicillin/streptomycin (Sigma), 9.05 mM $NaHCO_3$ (Synthi), and 20 mM HEPES (Sigma) [23]. Subsequently, the tissue was fixed with 4 % paraformaldehyde (Sigma) for 2 h at room temperature and washed six times for 10 min with phosphate-buffered saline (PBS, pH 7.4). Then, the tissues were embedded in a freezing medium (Tissue-Tek) and cut in a cryostat (Leica). On glass slides, the tissues following were permeabilized with PBS supplemented with 1 % Triton X-100 for 3 h at room temperature. For primary incubation, the GIP and GLP-1 antibodies were used at 1:3,000 dilution in PBS plus 1 % Triton X-100 overnight. Afterwards, the slices were washed with PBS plus 1 % Triton X-100 for 2 h and divided into six washes for 20 min. Finally, the slices were incubated in the dark for 1 h at 37 °C with the secondary antibodies diluted at 1:500 in PBS plus 1 % Triton X-100, conjugated with fluorescein isothiocyanate (FITC) for GIP (green fluorescence) and rhodamine for GLP-1 (red fluorescence). The slices were washed for 2 h, and soon, the laminas were mounted with UltraCruz™ Mounting Medium. The image was observed under a fluorescence microscope (Olympus IX70). Quantification of immunohistochemical content was assessed by the Cell-M program and was expressed in arbitrary values.

Statistical Analysis

Statistical analysis of anthropometric and biochemical parameters of OB NDM2 and T2DM patients were calculated using the independent-samples *T* test with representation of standard deviation (±SD). Glucose levels only were calculated using the nonparametric Mann-Whitney *U* test, with 95 % confidence interval. Mean glucose levels in the blood pre- and postsurgery were compared using the paired-samples *T* test with a significance value of $p \leq 0.05$.

Exponential median values for each gene mRNA expression and immuno content, in the jejunum, were compared

between the two patient groups using the nonparametric Mann-Whitney *U* test, with 95 % confidence interval.

Results

Individuals who participated in the study were divided into two groups: NDM2 and T2DM, characterized in Table 2. All analyses quantify the mRNA expression in the jejunum of these patients. We observed no statistical difference in mRNA expression of proglucagon, GIP, and DPP-IV between obese NDM2 and T2DM patients (Table 3). However, the obese T2DM subjects presented significantly low PC1/3 mRNA expression, when we analyzed this expression with both primers. Many patients (36.5 %) did not expressed mRNA of PC1/3 at these conditions. We confirm by gene sequencing that the cDNA generated in the jejunum of obese NDM2 and T2DM patients is 99 % similar with human PC1/3 variables.

The study of eutrophic individuals demonstrated that the jejunum at 30 cm from the Treitz ligament expresses only GIP (median 0.641 (0.061–119.19) and DPP-IV (median 1.577 (0.004–25.99) mRNA.

Immunohistochemistry revealed intracellular presence of GIP and GLP-1 in the jejunum sections 60 and 100 cm distal of the ligament of Treitz in both patient groups. Only the cells K and L were immunoreactive for GIP and GLP-1, respectively (Fig. 1). Moreover, GIP and GLP-1 immuno contents of the jejunum of T2DM patients revealed to be significantly less compared those of NDM2 patients' jejunum (Table 4).

As shown in Table 5, high glucose levels stimulated PC1/3 mRNA expression, in the jejunum section in T2DM. Also, after incubation with high glucose, the immuno contents of GLP-1 jejunum cells of T2DM patients increased significantly (Table 4). We observe that the values reach like those of NDM2 subjects. The immuno contents of GIP increased significantly in the NDM2 group after 2 h of glucose incubation, but remained low in the T2DM group.

Discussion

Obesity is a pathological state that involves other comorbidities, in particular T2DM [25]. OB and T2DM individuals have shown an immediate improvement in the levels of glucose after the surgical procedure, which allowed a reduction in the administration of antidiabetic drugs [1, 2, 4, 26–28] before weight loss [9]. There is an anatomical change in the gastrointestinal tract of individuals who undergo surgery, and this would involve the secretion of peptides associated with insulin secretion, such as GIP and GLP-1 [29]. After surgery, the jejunum receives previously ingested food, and we assume that this will stimulate cells for transcription, synthesis, release, and inactivation of incretins.

Table 2 Anthropometric and biochemical parameters of morbidly obese NDM2 and T2DM patients

Data	NDM2 group (n=20)	T2DM group (n=18)
Age (years)[a]	33.88±9.84	46.67±10.23
BMI (kg/m^2)[a]	50.43±9.54	53.41±8.37
Waist (cm)[a]	135.64±14.31	140.89±15.62
Hip (cm)[a]	145.36±11.28	150.89±12.97
HDL (mg/dl)[a]	48.25±12.83	51.33±15.23
LDL (mg/dl)[a]	112.43±28.91	124±38.12
TG (mg/dl)[a]	136.5±62.44	174.67±43.41
CT (mg/dl)[a]	188.69±31.33	215.44±37.46
Glucose (mg/dl)[b]	92.5 (85.42–99.69)	111 (83.8–206.4)
Insulin(μU/ml)[a]	44.1±45.88	27.6±12.01

[a] Data show mean±SD

[b] Data show median (95 % confidence interval)

It is related that OB patients present low release of GLP-1 compared with NOB patients, when the jejunum has contact with the food [3, 30, 31]. Therefore, our team of surgeons removed 60- and 100-cm sections of the bowel of obese NDM2 and T2DM patients, respectively, in order to improve the secretion of incretins, especially GLP-1. It is proposed that this procedure improves T2DM clinical parameters [3, 10]. Initially, in basal conditions, we observed that the jejunum mucosal cells of NDM2 and T2DM patients expressed no different relative proglucagon, GIP, and DPP-IV mRNA levels. Thus, diabetes control cannot be justified by the mRNA levels of proglucagon, GIP, and DPP-IV in proximal jejunum cells, or else, this is not justified by the transcriptional

Table 3 GIP, proglucagon, DPP-IV, and PC1/3 mRNA expression in the jejunum of morbidly obese NDM2 and T2DM subjects

Gene	NDM2 (n=20)	T2DM (n=18)	P value
GIP	1.9 (0.01–37.56) n=17	1.22 (0.10–33.73) n=12	NS
Proglucagon	0.8 (0.2–16.27) n=14	1.6 (0.08–5.24) n=13	NS
DPP-IV	3.42 (0.001–49.19) n=16	1.99 (0.08–14.03) n=15	NS
PC1/3 a	1.06 (0.45–21.01) n=8	0.2 (0.14–4.74) n=7	p=0.014
PC1/3 b	1.02 (0.78–1.25) n=3	0.55 (0.17–0.92) n=6	p=0.048

Sections 60 and 100 cm from the Treitz ligament were obtained from obese NDM2 and T2DM patients, respectively. Samples were collected during surgery and were immediately immersed in TRIzol reagent. To compare the expression pattern of mRNA in the jejunum of obese NDM2 and T2DM subjects, figures were normalized against β-actin (ΔCt) and calibrated against the mean of NDM2 mRNA patients ($2^{-\Delta\Delta Ct}$). To compare NDM2 and T2DM, p values were calculated using the nonparametric Mann-Whitney *U* test. Values were expressed as median and 95 % confidence interval. Statistical significance was at $p \leq 0.05$

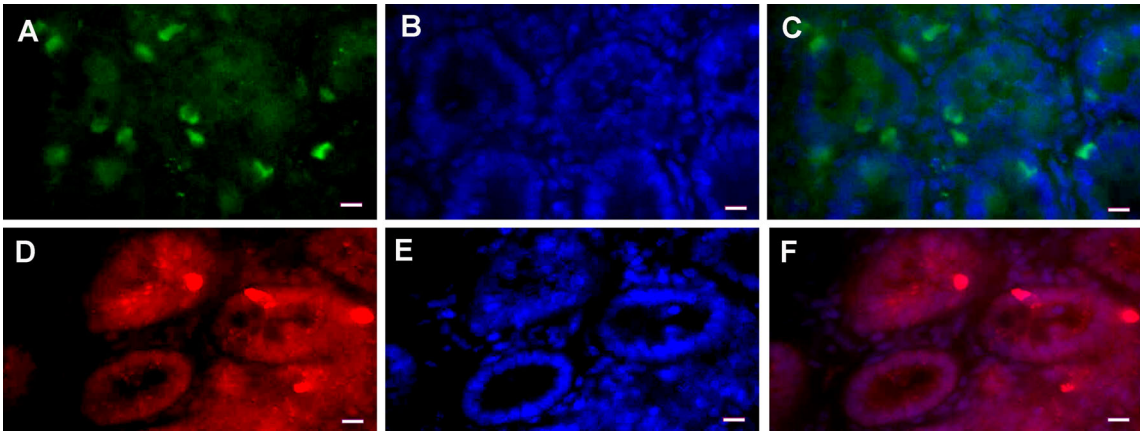

Fig. 1 Representative images of the presence of GIP and GLP-1 in mucosa cells of the jejunum of OB and NDM2 individual. All images were captured with a magnification of × 20. Sample processing is described in "Materials and Methods." **a** and **d** represent the fluorescent marking of GIP (*green*) and GLP-1 (*red*), respectively, within the endocrine granules. **b** and **e** show labeling in the nuclei (*blue*). **c** and **f** demonstrate the merged images: **a** and **b**, **d** and **e**. *Scale* 50 μm

control of these genes. However, the bioactivation of incretins may be the focus of investigation because approximately 39 % of all patients do not express PC1/3 mRNA in the jejunum. We confirmed by gene sequencing that the cDNA generated in the jejunum of obese NDM2 and T2DM patients was 99 % similar to human PC1/3. Failure to detect any signal in other patients may be the result of mutations in the annealing region of the primer or of other mRNA gene processing of PC1/3. A gene mutation codifying a human congenital PC1/3 deficiency has been described; the syndrome is characterized by obesity and small intestine dysfunction [15, 32–35]. Jackson et al. [34, 35] and Frank et al. [31], suggested that this gene mutation caused a failure of enzyme maturation. These results show that PC1/3 has an important role in human metabolic and endocrine disease. Decreased signal or mutation of PC1/3 could play a key role in the pathophysiological basis of incretin deficiency in obese T2DM patients, because this enzyme is responsible for the bioactivation of proglucagon and GIP precursor. In addition, T2DM patients with a functional gene expression showed a decreased PC1/3 mRNA expression in basal conditions compared to those with NDM2. This fact may explain a posttranslational control of GLP-1 and GIP levels in diabetic patients. In analyzing mRNA expression of eutrophic subjects, it was only the expression of GIP and DPP-IV was observed. There was no signal for proglucagon and PC1/3 mRNA in this region of the jejunum. This is a molecular justification to the surgical cut to be greater than 60 cm distal of the ligament of Treitz.

Table 4 Immuno content of GIP and GLP-1 in jejunum cells of obese NDM2 and T2DM patients, at time 0 and after incubation with or without high glucose for 2 h

	$t=0$	Control	High glucose
GIP			
NDM2	59.3 (47.0–97.0)	93.43[a] (73.84–115.88)	122.5[a,b] (107.5–153.7)
T2DM	45.54[c] (29.16–61.2)	57.7[a,c] (35.2–153.3)	57.5[c] (27.9–172.7)
GLP-1			
NDM2	71.83 (51.4–133.4)	98.8 (74.13–114.13)	119[a] (71.5–164.5)
T2DM	49.08[c] (37.10–70)	66.03[a,c] (42.23–96.27)	82.22[a,b,c] (46.87–123.08)

Sections at 60 and 100 cm from the Treitz ligament were obtained from obese NDM2 ($n=20$) and T2DM ($n=18$) patients, respectively. Proximal jejunum samples collected during surgery were analyzed at time 0 ($t=0$) either incubated with or without 11 mM glucose for 2 h. Each analysis was performed in triplicate. After, the tissues were fixed with paraformaldehyde, embedded in a freezing medium, cut in a cryostat, and incubated with primary and secondary antibodies (see "Materials and Methods"). The secondary antibodies were conjugated with FITC for GIP (green fluorescence) and rhodamine for GLP-1 (red fluorescence). The image was observed under a fluorescence microscope (Olympus IX70). Quantification of immunohistochemical content was assessed by the Cell-M program, and it was expressed in arbitrary values. To compare glucose treatment with control and $t=0$, p values were calculated using the nonparametric Mann-Whitney U test. Values were expressed as median and 95 % confidence interval. Statistical significance was at $p \leq 0.05$

[a] Statistically different from $t=0$

[b] Statistically different from control

[c] Statistically different from NDM2

Table 5 GIP, proglucagon, DPP-IV, and PC1/3 mRNA expression in the jejunum of obese NDM2 and T2DM patients after incubation with or without high glucose for 2 h

Gene		Control	High glucose
PC1/3	NDM2	0.85 (0.10–10.16)	0.6 (0.3–0.6)
	T2DM	0.95 (0.41–1.51)	15.69 (6.47–21.16)*
DPP-IV	NDM2	1.22 (0.10–39.77)	0.25 (0.11–0.70)
	T2DM	0.79 (0.28–1.66)	1.94 (0.19–8.53)
Proglucagon	NDM2	0.74 (0.12–18.87)	0.43 (0.29–0.76)
	T2DM	1.21 (0.16–2.01)	0.69 (0.33–0.98)
GIP	NDM2	0.9 (0.10–13.12)	1.24 (0.33–2.22)
	T2DM	1.5 (0.23–2.36)	0.43 (0.09–0.74)

Sections at 60 and 100 cm from the Treitz ligament were obtained from obese NDM2 (n=20) and T2DM patients (n=18) patients, respectively. Proximal jejunum samples collected during surgery were incubated with or without 11 mM glucose (see "Materials and Methods"). Each treatment was incubated in triplicate. After incubation, the medium was removed and TRIzol was added for RNA extraction. To compare the expression pattern of mRNA in the jejunum of obese NDM2 and T2DM subjects, figures were normalized against β-actin (ΔCt) and calibrated against the mean of control treatment mRNA ($2^{-\Delta\Delta Ct}$). To compare glucose treatment and control, p values were calculated using the nonparametric Mann-Whitney U test. Values were expressed as median and 95 % confidence interval

*$p \leq 0.05$ (statistically significant)

It has already been reported that bariatric surgery normalized glucose serum levels in T2DM patients [4, 36, 37], and this was observed in our patients 3 months later. After surgery, the food acts directly on cells of the jejunum located 60–100 cm from the ligament of Treitz. Thus, food components, such as glucose, can modulate the synthesis and release of incretins. We observed that higher concentrations of glucose (11 mM) significantly increased in vitro the mRNA expression of the enzyme PC1/3 in jejunum cells. This could explain the major GLP-1 secretion after surgery in T2DM patients and the improvement of glycemia [3, 4, 22]. This could be by PC1/3 bioactivation of GLP-1, which stimulates insulin secretion, through the entero pancreatic axis [38].

Quantification of intracellular immuno contents, at the moment of surgery, proved that T2DM patients present less bioactive GIP and GLP-1 than those with NDM2. This result corroborates with the low PC1/3 mRNA expression found in these patients at this time. The in vitro effect of high glucose in jejunum cells demonstrated an upregulation of the enzyme responsible for the bioactivation of GIP and GLP-1. In our study, jejunum cells stimulated with high glucose increased PC1/3 mRNA expression and intracellular content of GIP and GLP-1 in NDM2 and T2DM patients, respectively.

At basal conditions, in sections 100 cm distal of the ligament of Treitz, jejunum cells received more macronutrients as fatty acids than glucose; in general, carbohydrate digestion and glucose absorption occur before they have passed beyond the duodenum and upper jejunum [30]. After surgery, digestion of carbohydrates increases glucose concentration in the gut region farther away. A new condition is established: the endocrine jejunum cells suffer new modulation.

In conclusion, obese T2DM presents decreased expression of PC1/3 mRNA in the jejunum that can interfere on the bioactivation of incretins. This low expression could be linked to this mutation or transcriptional control. We suggest that after surgery, T2DM normalizes the GLP-1 levels and glycemia through the direct action of diet components (glucose) on the modulation of PC1/3 in proximal jejunum cells. However, the present study has a limitation: the jejunum sections of the three patient groups were collected at different levels. Therefore, the results could be associated to different cellular distributions of endocrine cells along the human gut. But, based on our findings, we assumed that the region of the human jejunum, from NDM2 and T2DM individuals (60 or 100 cm far from the Treitz ligament, respectively) contains L and K cells because the tissue expressed proGIP and proglucagon mRNA, and we detected immuno content of both insulinotropic peptides. The results suggest that PC1/3, coexpressed with ProGIP and proglucagon in the K and L cells, could determine a difference between the jejunum of the NDM2 and T2DM individuals. In eutrophic patients, the expression of GIP and DPP-IV alone, in the upper small intestine, reveals abundant occurrence of K cells in this region. Further studies are needed to investigate PC1/3 polymorphism for obesity treatment and to clarify the downregulation of PC1/3 expression in diabetic individuals and the nonexpression of this enzyme in some obese individuals.

Acknowledgments We thank CNPq for the financial support.

Conflict of Interest The authors declare no conflict of interest.

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