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Dissertação - Artigo de investigação médica

Mestrado Integrado em Medicina – ICBAS-UP

Incretin cell distribution at gastro-enteric anastomosis of gastric bypass is variable depending on the biliopancreatic limb length in type 2 diabetics

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Porto 2017

Acknowledgments:

Em primeiro lugar, gostaria de agradecer à minha orientadora Professora Mariana Monteiro, que confiou em mim para abraçar este projeto de investigação que durou 4 anos e que me deu a oportunidade de trabalhar como um verdadeiro "cientista". Não só a Professora foi um marco nas minhas aulas de Anatomia, mas também é um exemplo de que muito dos nossos sucessos são alcançados com muita dedicação e persistência!

Em segundo lugar, queria agradecer à minha co-orientadora Sofia Pereira, que esteve sempre comigo nesta montanha russa. Obrigado Sofia por acreditares sempre em mim e por me motivares nos melhores e sobretudo nos piores momentos. O teu carinho, a tua boa disposição e o teu apoio são, sem dúvida, uma inspiração que eu levo para a minha vida!

Em terceiro lugar, gostaria de agradecer à Madalena pelo companheirismo, pelo trabalho de equipa e por todo o espírito que vivemos no laboratório.

Obrigado ao Dr. Mário Nora e à Prof. Doutora Marta Guimarães pela disponibilidade em contribuir para o meu trabalho fornecendo as amostras para esta investigação.

Gostaria também de deixar um agradecimento ao Professor Artur Águas, que me acolheu no seu departamento de Anatomia, um exemplo de enorme competência e excelente ambiente entre todos! É fantástico poder dizer que esta é minha segunda casa! Obrigado Ana pelos banhos de sol e pelos patins em linha à espera dos resultados das imuno. E um obrigado ao Tiago por estar sempre disponível para ajudar e por fazer os melhores roteiros gastronómicos.

Um agradecimento ao André Maia por toda a disponibilidade e empenho para analisar os meus dados de imunofluorescência e à Ângela por ter sido incansável em acompanhar-me nas cirurgias no Hospital São Sebastião.

Queria também deixar um especial agradecimento à minha querida Mãe – tudo o que atingi nos meus 23 anos de vida devo-lhe a si. Obrigado Mãe por amar-me como ninguém e ter abdicado do que jamais alguém abdicaria por mim! Os meus sucessos são os seus sucessos e não podia estar mais grato por isso.

Obrigado Pai por seres um exemplo de sucesso em tudo o que fazes e por me fazeres ver que o trabalho árduo acaba por compensar. Obrigado por exigires sempre mais de mim e por quereres que seja uma pessoa excecional em todas as dimensões. Queria também agradecer aos meus avós que são um exemplo do amor, bondade e união que raramente se vê hoje em dia. É com uma enorme felicidade que estão aqui para presenciarem o nascimento do 2º médico na família. Também gostaria de agradecer aos meus outros avós que infelizmente não estão cá mas que os levo eternamente no meu coração.

E por último, à minha melhor amiga Catarina, a irmã que nunca tive com quem partilho a minha vida há mais de 13 anos todos os dias. À nossa amizade que é das coisas mais genuínas que existem!

Resumo:

Introdução: As hormonas incretinas desempenham um papel importante na melhoria da diabetes tipo 2 (T2D) após o bypass gástrico Y em Roux (RYGB). As células secretoras das incretinas polipéptido insulinotrópico dependente da glucose (GIP) e o péptido glucagão-like 1 (GLP-1) estão distribuídas distintamente ao longo do intestino delgado humano. O nosso objetivo foi avaliar se aumentando o comprimento da ansa biliopancreática no RYGB nos pacientes com diabetes tipo 2 modificaria a proporção relativa de células secretoras de incretinas encontradas ao nível da anatomose gastro-entérica.

Métodos: Fragmentos de intestino delgado (n=38) foram colhidos após secção intestinal realizada a duas distâncias diferentes a partir do ângulo duodenal durante o RYGB; entre os 60-90 cm (n=27) de pacientes com T2D (n=11) e não diabéticos (n=16) ou aos 200 cm (n=11) de pacientes com T2D. As células secretoras de GIP e GLP-1 foram marcadas por imunohistoquímica e quantificadas medindo a percentagem de área marcada com um programa morfométrico computorizado enquanto a marcação por imunofluorescência de células co-secretoras de GIP/ GLP-1 foram quantificadas usando um sistema de análise de células.

Resultados: No intestino delgado proximal, o padrão de distribuição das células produtoras de incretinas na mucosa dos pacientes com T2D foi semelhante à dos pacientes não diabéticos. No entanto, a distribuição destas células aos 200 cm a partir do ângulo duodenal de pacientes T2D revelou uma densidade de células produtoras de GIP significativamente mais baixa (26.44 ± 2.85 vs. 17.50 ± 1.54, p=0.015) e uma densidade de GLP-1 semelhante quando comparada com a mucosa proximal. A distribuição de células co-marcadas por GIP/GLP-1no intestino proximal de pacientes T2D e não

diabéticos foi semelhante bem como quando comparada com a mucosa intestinal proximal e de T2D.

Conclusões: Em pacientes T2D, a densidade relativa de células produtoras de incretinas aos 200 cm a partir do ângulo duodenal é significativamente diferente comparativamente com a mucosa proximal, sendo que uma ansa biliopancreática mais longa no RYGB produz um padrão distinto de células produtoras de incretinas ao nível da anastomose gastro-entérica que poderá potencialmente resultar em perfis endócrinos e resultados metabólicos diferentes.

Palavras-chave: GLP-1, GIP, incretinas, bypass gástrico, T2D, obesidade

Abstract:

Background: Incretin hormones play an important role in type 2 diabetes (T2D) improvement after Roux-en-Y gastric bypass (RYGB). Glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) incretin secreting cells are distinctively distributed along the human small gut. Our aim was to assess whether increasing the length of RYGB biliopancreatic limb in T2D patients would modify the relative proportion of intestinal incretin secreting cells found after the gastric outlet.

Methods: Small intestine biopsies (n=38) were harvested after intestinal section performed at two different distances from the duodenal angle during RYGB; either at 60-90 cm (n=27), from T2D (n=11) and non-diabetic (n=16) patients, or at 200 cm (n=11) from T2D. GIP and GLP-1 secreting cells were immunohistochemistry-stained and quantified by measuring the percentage of stained area with a computerized morphometric tool, while GLP-1/GIP immunofluorescence co-staining cells were quantified using a cell analyzer system.

Results: At the proximal small intestine, the pattern of incretin cell distribution in the mucosa of T2D patients was similar to non-diabetic individuals. However, the incretin cell distribution at 200 cm from the duodenal angle of T2D patients depicted a significantly lower GIP cell density (26.44±2.85 vs 17.50±1.54, p=0.015) and a similar GLP-1 density (1.67±0.26 vs 1.62±0.20, p= 0.88) when compared to proximal mucosa. GIP/GLP-1 co-staining cell distribution in the proximal intestine of T2D and non-diabetic patients was similar, as well as in the proximal and distal intestinal mucosa of T2D.

Conclusion: In T2D patients, incretin cell relative density at 200 cm from the duodenal angle is significantly different from the proximal mucosa, thus RYGB a longer biliopancreatic limb produces a distinctive incretin cell pattern at the gastro-enteric

anastomosis, which could potentially result in different endocrine profiles and metabolic outcomes.

Keywords: GLP-1, GIP, incretin cells, gastric bypass, T2D, obesity

Table of contents:

Introduction:

Glucose-dependent insulinotropic polypeptide (GIP) and glucagon like peptide (GLP-1) are enteroendocrine peptide hormones that are responsible for the 50-70% additional insulin secretion elicited by oral glucose administration when compared to intravenous glucose infusion, the well-known incretin effect [1, 2]. GIP and GLP-1 expressing cells are distinctively distributed along the small gut, as GIP is secreted by K cells predominantly located in the duodenum and proximal jejunum [3], while GLP-1 is produced by the L cells mostly found in the distal jejunum and ileum but also in pancreatic alpha-cells [4]. More recently, a cell population co-secreting GIP/GLP-1 has also been described [5].

GLP-1 besides stimulating glucose-dependent insulin release [6] was also demonstrated to promote beta-cell proliferation *in vitro* and *in vivo* in rodents [7], suppress hepatic glucose output by inhibiting glucagon secretion [8], inhibit food intake and gastric emptying [9] and have cardio-protective functions [10]. On the other hand, GIP despite having no significant effects on gastric emptying, food intake or body weight [11], in addition to the incretin effect seems to have other peripheral functions particularly in fat tissues by promoting lipid accumulation in subcutaneous adipocytes [12, 13] and also in the skeleton by increasing bone formation [14].

A decreased incretin effect is an early feature of T2D, presumably as a consequence of GIP resistance [15] and impaired GPL-1 secretion despite retained insulin tropic activity [16, 17] Pharmacological administration of GLP-1 was demonstrated to increase insulin secretion and decrease glucose levels [18], thus GLP-1 analogues were developed and are now well established for diabetes treatment [19]. In contrast to the well-established pharmacological use of GLP-1 analogues, the physiological role GIP as well as in the

glycemic dysregulation observed in T2D is less clear. The pharmacological effects of GIP analogues were shown to stimulate glucagon secretion while did not affect significantly insulin secretion, which has led to a lack of enthusiasm on the perspective of the use of this peptide for T2D treatment [20].

Several bariatric surgery procedures proved to be more efficient than conventional medical treatment in improving glucose control and inducing prolonged T2D remission in obese patients [21]. The anti-diabetic effect of bariatric surgery has been partially attributed to the changes in gastrointestinal hormones triggered by the anatomical rearrangement of the gastro-intestinal tract that were widely documented [22]. In particular, the increase in GLP-1 response observed after some bariatric surgical procedures seems to have a significant role in inducing diabetes metabolic improvement or even clinical remission, and is believed to be a consequence of early stimulation of L-cell at the gastro-enteric anastomosis by undigested nutrients brought [23]. In addition to this possible mechanism, the "foregut hypothesis" proposes an alternative explanation for the phenomena and states that the exclusion of the proximal small intestine from the gastro-intestinal transit could avoid the release of putative anti-incretin factors that to be identified [24]. Although the two hypothesis are not mutually exclusive as both anatomical rearrangements concur after the bariatric procedures with proven anti-diabetic effects, the relative contribution or the underlying endocrine mechanisms still need clarification.

In addition, T2D remission rates after RYGB with longer biliopancreatic limbs was reported to be higher as compared to classic procedures with biliopancreatic limbs of 60-100 cm [25]. Despite limited data with head-to-head comparisons, these results could potentially be explained by a different arrangement of the intestinal anatomy inducing variable gut hormone secretion profiles [22]. In support of this hypothesis is the work of

Guedes *et al* that documented the distinctive distribution of incretin secreting cells along the human small intestine of 30 cadavers, demonstrating a striking change in the GIP and GLP-1 cell density in between 80 to 200 cm from the duodenal angle [26]. In order to understand whether there is an anatomical substrate to support the modification of the surgical procedure with the rationale of improving the endocrine and metabolic profiles of diabetic patients, we sought to analyze whether there were differences in incretin cell pattern between proximal and distal small intestine of obese T2D patients undergoing RYGB.

Therefore, our aim was to compare the incretin secreting cell distribution in the small intestine at the level of the gastro-enteric anastomosis of T2D and non-diabetic patients. Our ultimate aim was to evaluate whether if increasing the length of the RYGB biliopancreatic limb in type 2 diabetic (T2D) patients would modify the relative proportion of incretin secreting cells found after the gastric outlet in order to be likely to produce a distinctive endocrine profile.

Material and methods:

Sample selection and histologic procedures

After approval by the institutional Ethical Committee, all patients received detailed explanations about the study protocol, accepted to participate and provided written informed consent for the surgeon to perform a small intestinal biopsy during elective laparoscopic gastric bypass procedure undertaken for the primary treatment of obesity and related co-morbid conditions at a single obesity treatment center.

Small intestinal fragments (n=38) comprising the full thickness of the small gut were harvested from the site of the intestinal section made as part of the routine procedure to perform the RYGB gastro-enteric anastomosis. These fragments were collected from two different intestinal sites located either between 60-90 cm (n=27) or at 200 cm (n=11) from the duodenal angle. The proximal intestinal fragments were collected form non-diabetic (n=16) and T2D (n=11) patients, whereas the distal intestinal fragments were only collected from patients with previous T2D diagnosis. Intestinal tissue was immersed in 4% buffered formaldehyde (Panreac®, Barcelona, Spain) immediately after removal, preserved for 72 hours and then routinely processed for paraffin embedding and optical microscopy.

Immunohistochemistry techniques

For incretin secreting K- and L-cell detection, immunohistochemistry (IHC) with anti-GIP (ab30679, Abcam, Cambridge, UK) and anti–GLP-1 (ab22625, Abcam) specific antibodies were used, respectively. Anti-chromogranin A (ab17064, Abcam) specific antibody was used as unspecific marker for neuroendocrine cell identification.

Briefly, 3μm formalin-fixed paraffin embedded tissue sections mounted on adhesive microscope slides (StarFrost, Knittel Glass, Germany) were deparaffinized, rehydrated in

graded alcohols and underwent heating by microwaving at 900 W in 10 mM citrate buffer (pH 6.0) with .05% Tween 20 during 18 minutes, for antigen retrieval. The endogenous peroxidase was blocked with a diluted solution of 3% hydrogen peroxide in methanol for 20 minutes, followed by incubation in a normal serum (1:5 in 10% BSA) for 30 minutes. All IHC techniques were performed in coverplates on a Sequenza rack (TermoScientific, Waltham, MA) to ensure a homogeneous distribution of the antibodies used. The incubation with the primary antibodies Mouse anti–GIP (1:500 dilution in 5% BSA), Rabbit anti-GLP-1 (1:4000 in 5% BSA), and Rabbit anti-chromogranin A (1:100 in 5% BSA) was performed overnight at 4ºC. For reaction detection, chromogranin A and GLP-1 slides were incubated for 30 minutes with biotinylated secondary antibody polyclonal swine anti-rabbit (1:200, EO35301-2, Dako, Glostrup, Denmark) and biotinylated secondary antibody polyclonal rabbit anti-mouse (EO35401-2, Dako) was used for GIP. Afterwards, the slides were incubated with the avidin-biotin complex (ABC) (1:100 dilution in 5% BSA; VectorLaboratories, Peterborough, UK) for 30 minutes at room temperature. Diaminobenzidine was used as chromogen (Dako), followed by nuclear staining with Harris hematoxylin.

Immunofluorescence techniques

For immunofluorescence detection, the procedures were similar to the previously described for the IHC with the sole exception of the use of Sudan black B 0,5% in 70% of alcohol for 3 minutes after the endogenous peroxidase blockade in order to decrease tissue self-fluorescence. The incubation was followed with normal serum (1:5 in 10% BSA) for 30 minutes and afterwards incubated with primary antibodies goat Anti-GIP (1:500 dilution in 5% BSA) and Goat Anti-GLP-1 (1:2000 in 5% BSA). For GIP/GLP-1 detection slides were incubated for 2 hours with a cocktail containing a fluorescent

secondary antibody goat anti-rabbit (1:1000 in 5% BSA) and goat anti-mouse (1:750 in 5% BSA). The slides were mounted and counterstained with DAPI hard set.

Computerized image analysis

Immunohistochemistry stained slides were scanned using the image acquisition software Olympus VS110 virtual slide scanning system. Images were analysed using an image processing software (ImageJ, National Institutes of Health, USA) with a colour deconvolution plugin which separates the stained area from the initial image allowing quantification of the percentage of the area specifically stained with the GLP-1, GIP and chromogranin A antibody. The percentage of stained area (%SA) within the small intestinal mucosa area for each given molecular marker, GIP, GLP-1 and Chromogranin A, was quantified. The ratio of the %SA for GIP/chromogranin A and GLP-1/chromogranin A, was calculated as a surrogate of the relative proportion of GIP and GLP-1 cells among the intestinal neuroendocrine cell population. The GLP-1/GIP ratio was calculated to estimate the relative proportion of the two cell populations.

For immunofluorescence analysis, GIP and GLP-1 co-stained microscope slides were scanned using an IN Cell Analyser 2000 system (GE Healthcare). Images were acquired with a Nikon 20x/0.45 Plan Fluor Objective spanning 2.0 um thickness of the sample. Image analysis was performed with IN Cell Investigator software (GE Healthcare). Briefly, the image analysis protocol consists in the identification of double positivity for GIP/GLP-1 through a fluorescence intensity-based segmentation. The percentage of GIP/GLP-1 cells was then obtained by the ratio between the total number of double positives and the total mucosa area and results expressed in megapixel (MP).

Statistical analysis

Variables are expressed as mean and standard error of the mean. The difference between two independent experimental groups was evaluated using the unpaired Student t test for normally distributed variables and the Mann-Whitney U test for variables that did not meet the normal parameters. A p value < 0.05 was considered statistically significant. All statistical analysis were performed with the aid of the Graphpad Prism software version 6.01 for Windows.

Results:

The small intestine samples obtained during laparoscopic RYGB surgery (n=38) were allocated into three different groups of patients according to the diabetes status and small intestinal location. These were collected at two different distances from the duodenal angle, either at 60-90 cm (n=27), from T2D (n=11) and non-diabetic (n=16) patients, or at 200 cm (n=11) from T2D.

Patients' demographics, anthropometric and clinical features among the different studied groups are depicted in Table 1 (**Table 1**).

Table 1 - Demographic, anthropometric and clinical characteristics of the individuals within each group

	Pre-surgery data		
	Non-diabetic proximal small gut	Diabetic proximal small gut	Diabetic distal small gut
Age (years)	40.65 ± 2.38	48.8 ± 2.07	48.58 ± 1.94
BMI (kg/m^2)	38.57 ± 1.14	40.02 ± 1.75	40.83 ± 1.01
Ratio F:M	13:3	7:4	7:5
Distance from duodenal angle (cm)	77 ± 3.60	87 ± 3.00	200 ± 0.00
Glycaemia (mg/dL)	105.5 ± 2.1	138.5 ± 5.4 ***	154.4 ± 14.1 ***
HbA1c (%)	5.2 ± 0.2	6.61 ± 0.2 ***	7.2 ± 0.5 ***
Insulin (pq/mL)	17.61 ± 4.21	17.55 ± 3.37	18.69 ± 3.57
HOMA-IR	4.46 ± 0.97	6.21 ± 1.27	7.09 ± 1.46
HOMA-B	166.50 ± 50.33	82.60 ± 15.28	85.59 ± 19.53

***p<0,001 vs non-diabetic; Non-significant differences were found between the two T2D groups.

There were no significant differences in patient baseline characteristics, namely gender distribution, age and mean BMI, except for the diagnosis of T2D and RYGB biliopancreatic limb length and also for the glycaemia and HbA1c that was significantly higher in the diabetic groups as compared with the normoglycaemic patients. There was a female predominance among all study groups, as a consequence of the unequal patient gender distribution among the ones seeking bariatric surgery in our center as previously reported by other series [27].

Immunohistochemistry was performed for evaluation of small intestinal mucosa stained with anti-GLP-1, anti-GIP and anti-chromogranin (**FIG. 1A, 1B, 1C**).

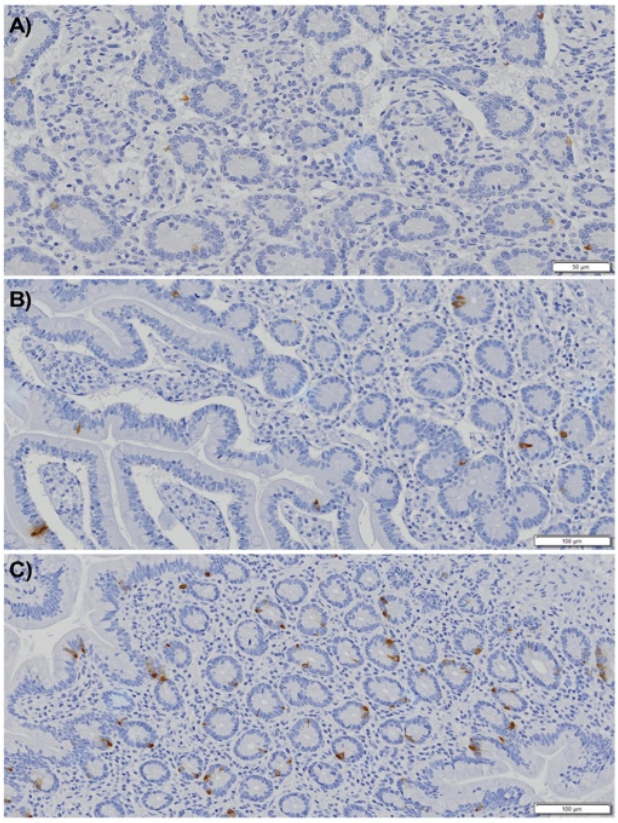

Figure 1 – Small intestine mucosa stained by immunohistochemistry with anti-glucagon-like peptide 1 (anti-GLP-1) (A); anti-glucose-dependent insulinotropic peptide (anti-GIP) (B) and anti-chromogranin A (C)

When comparing the incretin expressing cell distribution in the proximal small intestine of non-diabetic and diabetic patients, no statistically significant differences were found in the %SA of GLP-1 or GLP-1/chromogranin A cells (2.11 ± 0.45 % vs 1.67 ± 0.25 % ; $p>0.05$), despite the % of staining was lower in the T2D group (**FIG. 2A**). No significant differences were observed in the % of SA for GIP or GIP/chromogranin A cells between non-diabetic and T2D groups at the proximal small intestinal location (non-diabetic: 29.67 ±3.50 % vs diabetic: 26.44 ± 2.85 %) (**FIG**. **2B**). The %SA of GLP-1/GIP ratio was also not significantly different in non-diabetic (4.73 ± 0.33%) and diabetics groups (6.44 ± 0.82 %; $p > 0.05$) (**FIG. 2C**).

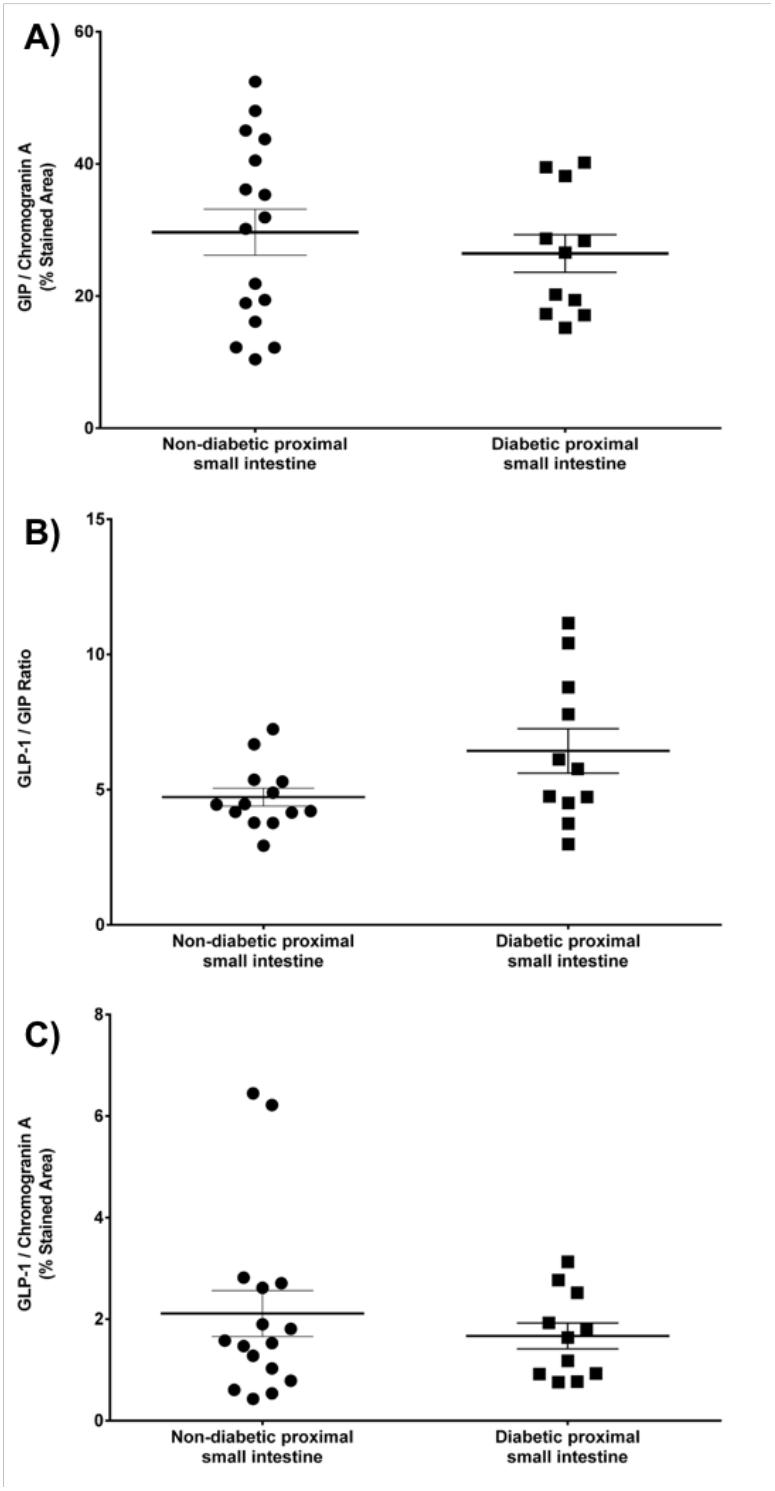

Figure 2 – Graphic representations of the percentage of stained area of GLP-1 (A), GIP (B) and GLP-1/GIP ratio (C) comparing proximal small intestine (60-90 cm) features from non-diabetic and T2D patients.

In T2D patients, when comparing proximal and distal small intestinal incretin secreting cell distribution, the % SA of GLP-1 and GLP-1/chromogranin A despite being numerically higher in the distal small intestine, this difference was not statistically significant (200 cm biliopancreatic limb: 1.62 ± 0.20 % vs 60-90 cm biliopancreatic limb: 1.67 ± 0.25 %; $p > 0.05$) (**FIG**. **3A**). However, the %SA of GIP and GIP/chromogranin A was significantly lower in distal small intestine (17.51 ± 1.54 %) as compared to proximal small intestine (26.44 ± 2.85 %, $p < 0.05$) (**FIG. 3B**). The %SA of GLP-1/GIP ratio of T2D patients was not statistically significant although higher in the distal small intestine as compared to the proximal location (10.86 ± 2.19 % vs 6.44± 0.82 %; $p = 0.053$) (**FIG**. **3C**).

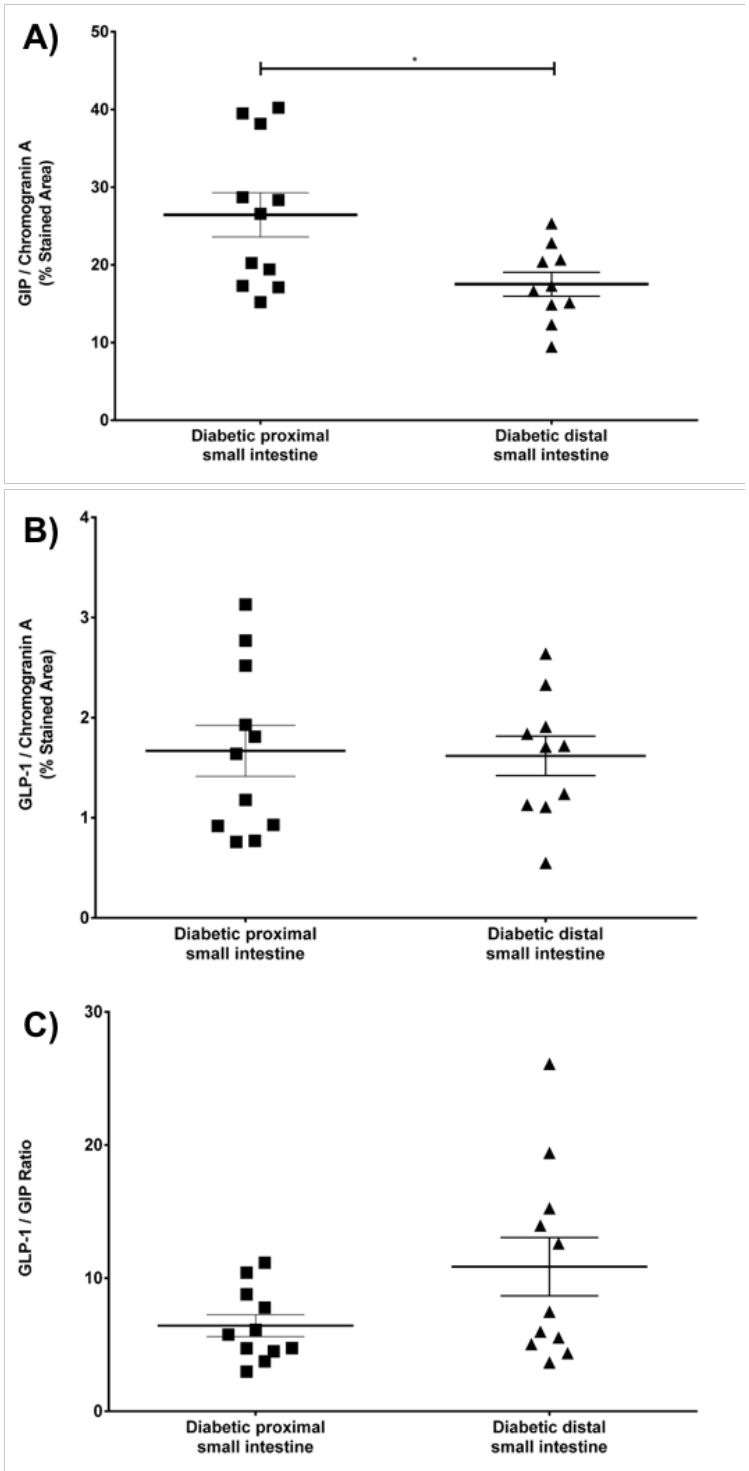

Figure 3 – Graphic representations of the percentage of stained area of GLP-1 (A), GIP (B) and GLP-1/GIP ratio (C) comparing the proximal small intestine (60-90 cm) and distal small intestine (200 cm) of T2D patients (* $p<0.05$)

Moreover, the absolute percentage of GIP secreting cells was significantly higher than GLP-1 secreting cells in both small intestinal locations and in both groups of patients. Co-staining GIP/GLP-1 cells distribution was found not to be significantly different between the two patient groups, although numerically higher in the non-diabetic patients as compared to T2D in the proximal (3.63 ± 0.71 % vs 2.43 ± 0.57 % MP) and distal small intestine (2.48 ± 0.43 %MP) (**FIG**. **4A, 4B, 4C**).

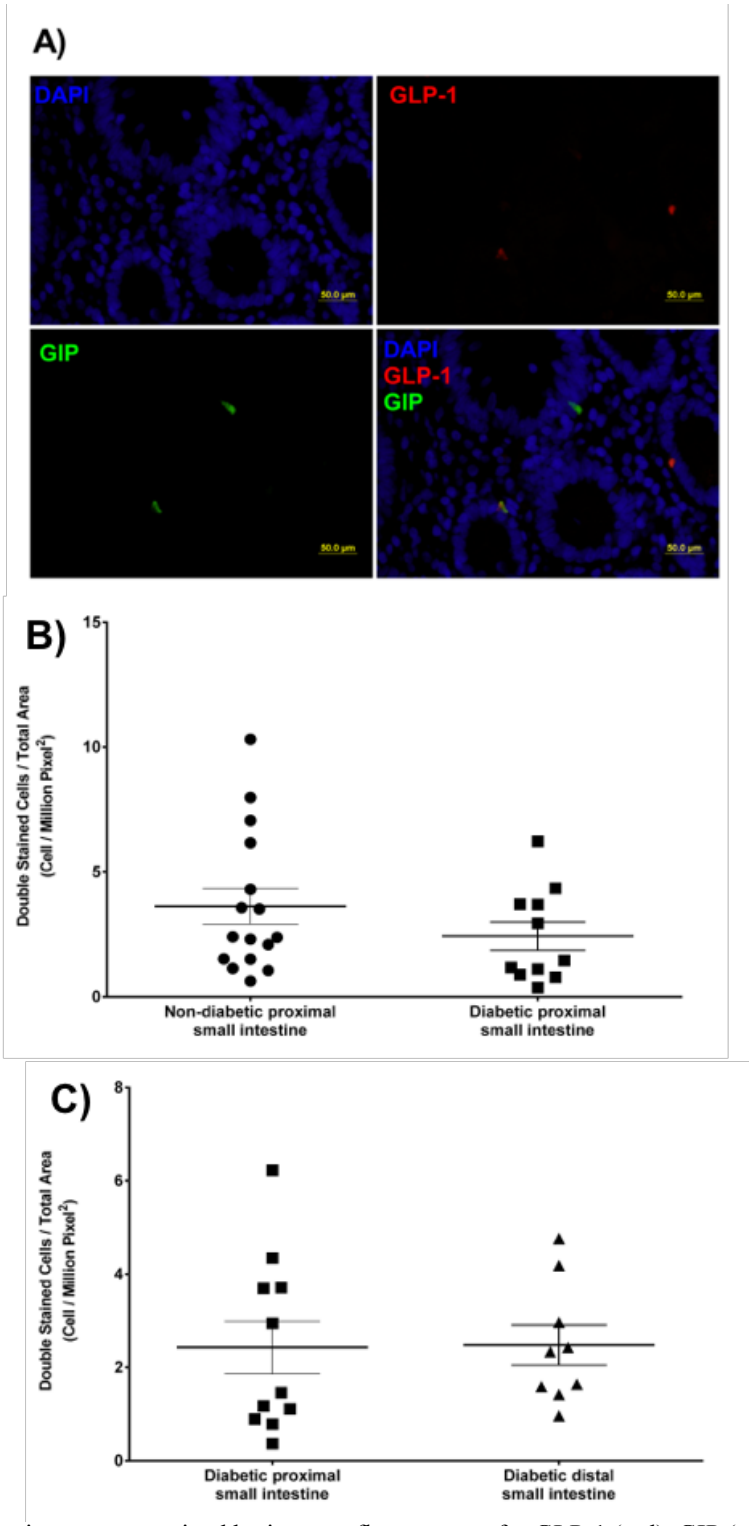

Figure 4 – Small intestine mucosa stained by immunofluorescence for GLP-1 (red), GIP (green) and DAPI (blue) (A) and graphic representation of co-stained cell in the three different studied groups non-diabetic(60-90 cm), T2D (60-90 cm) and T2D (200 cm) (B,C).

Discussion:

In this study, we compared for the first time GIP and GLP-1 expressing cell distribution at two different small intestinal locations, using biopsies harvested during RYGB surgery of non-diabetic and diabetic patients. For this, immunohistochemistry and immunofluorescence staining to assess GIP/GLP-1 co-localization were evaluated with the aid of a computerized morphometric analysis tool to allow a consistent, objective and unbiased quantification.

The percentage of GLP-1/chromogranin A, GIP/chromogranin A and GLP-1/GIP in the proximal small intestine located between 60 to 90 cm from the duodenal angle or the Treitz ligament were not found to be significantly different when diabetic and non-diabetic patients where compared. However, the distal small intestine of T2D patients depicted a distinctive incretin cell distribution when compared to the proximal small intestine with decreased GIP expression and increased GLP-1/GIP ratio.

T2D individuals are known to have significantly lower fasting GLP-1 plasma levels when compared to healthy individuals [28]. The meal-related glucagon-like peptide-1 response in type 2 diabetes is also decreased [29]. This overall decrease in GLP-1 is one of the possible reasons for the decreased incretin effect observed in T2D patients, as exogenous administration of GLP-1 is able to normalize fasting hyperglycemia and reduce postprandial glycemic increments [30].

Bariatric surgery is the most effective treatment for obesity that also improves glycaemic control, often within days after surgery, independently of weight loss. Bariatric surgery has even proved to be more effective than conventional medical therapy in inducing glycemic control, decrease diabetes co-morbidities and prevent mortality [21, 31]. One of the putative mechanisms leading to these anti-diabetic effects of bariatric surgery is the modification of gut hormone profiles [32]. In particular, RYBG is associated with

improved insulin secretion and an exaggerated postprandial rise in glucagon-like peptide 1 [33]. More recently, T2D remission rates after RYGB with longer biliopancreatic limbs were shown to be greater as compared to classic procedures with shorter biliopancreatic limbs of 60-100 cm [25]. In our own series the metabolic outcomes of T2D submitted to RYGB with a longer biliopancreatic limb, were found to be better than predicted from the previously reported for classic procedures [34]. A possible explanation for the enhanced anti-diabetic effects could be a different arrangement of the intestinal anatomy leading to distinctive gut hormone variations [22]. Guedes *et al* documented in 30 cadavers that GIP expressing cells were predominant in the first 80 cm after the duodenal angle, while GLP-1 expressing cells relative density was higher from 200 cm onwards [26]. Therefore, bariatric procedures that bring close to the gastric outlet a subset of intestinal mucosa with a higher density of GLP-1 expressing cells would be more likely to induce a distinct endocrine profile with increased GLP-1 secretion and further contribute to glycemic improvement.

To gain further insight whether modifying the classical RYGB procedure by increasing the biliopancreatic limb length would bring small intestinal mucosa with a distinctive incretin distribution to the level of the gastro-enteric anastomosis, we decided to analyze the relative differences in neuroendocrine cell distribution between the proximal (60-90 cm) and distal (200 cm) small intestine. We found that the percentage of GLP-1/chromogranin A, although not statistically different, was higher in the distal fragments as compared to the proximal ones, in support of the "hindgut hypothesis" [23].

Moreover, in T2D patients the percentage of GIP/chromogranin A was significantly lower in the distal small intestinal as compared to the proximal intestinal mucosa. GIP secretion after a glucose or mixed-meal load is exaggerated among diabetic patients. Moreover, GIP levels correlate with fasting and post-prandial triglyceride levels [35].

GIP receptors expression, in addition to pancreatic β-cells, is also found in other extra pancreatic tissues, such as the adipose tissue [12] having an active role in fat metabolism [12]. High-fat diet leads to a chronic stimulation of GIP secretion and promotes K cell hyperplasia in obese hyperglycemic mice [36]. In addition, McClean *et al* and Gault *et al* using a GIP receptor antagonist in high-fat-fed mice, (Pro^3)GIP, documented a significant body weight loss, amelioration of insulin resistance and improvement of glucose tolerance [37, 38]. Thus, GIP ablation was suggested as a potential target for obesity and diabetes treatment. GIP levels also decrease significantly after biliopancreatic diversion and RYGB surgery in T2D patients [33, 39] The significant lower GIP expression found in the small gut located at 200 cm from the duodenal angle in T2D patients, further supports the hypothesis that GIP exclusion, as achieved with longer biliopancreatic limbs, could contribute to explain the improved metabolic profiles as compared to classical procedures [24].

At last, we herein report the identification of GIP/GLP-1 co-secreting cells in a large subset of human small intestinal samples. This data further supports of the evidence that these two incretins can be secreted at the same cell so-called K/L cells, as previously described by Mortensen *et al* that analyzed small gut samples of three subjects [5]. We found no significant differences in the pattern of K/L cell distribution between the proximal and distal small intestine, or when diabetic and non-diabetic patients were compared. Thus, these results suggest that K/L cells might have a more homogenous distribution along the small gut as compared to GIP and GLP-1 secreting cells, while the role of this recently characterized cell population remains to be established [5, 40].

One of the main limitations of our study was the fact that we do not have distal small intestinal biopsies from non-diabetic patients, which would be most useful to assess how the relative incretin cell distribution in the small gut found in T2D patients compares to

normoglycemic individuals. However, to perform such a modified procedure in non-diabetic patients in order to collect an intestinal biopsy was not considered ethical nor granted approval.

In conclusion, incretin cell distribution at 200 cm from the duodenal angle is significantly different from the proximal small intestinal mucosa, as demonstrated in this subset of biopsies from T2D patients. Thus, a RYGB with a longer biliopancreatic limb results a distinctive incretin cell pattern at the level of the gastro-enteric anastomosis, which could lead to a different profile of gastro-intestinal hormone secretion and metabolic outcomes.

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Appendix 1 – Institutional Ethics Committee Deliberation

Centro Hospitalar
de Entre o Douro e Vouga, E.P.E.

Exmo. Senhor
Dr. Mário Nora
Diretor do Serviço de Ortopedia
Centro Hospitalar de Entre o Douro e Vouga, EPE

CA-0803/16-0c **Data:** 2016/06/15
MP/AC

Assunto: Estudo – "Bypass gástrico na Diabetes Mellitus Tipo 2: Impacto do tamanho da ansa alimentar na remissão DM2."

O Conselho de Administração do Centro Hospitalar de Entre o Douro e Vouga, EPE, apreciou o parecer elaborado pela Comissão de Ética relativo ao estudo mencionado em epígrafe, tendo proferido o seguinte despacho: *"Deliberado autorizar."*

Com os melhores cumprimentos,

Miguel Paiva
Presidente do Conselho de Administração