

Gut Peptide Hormones and Pediatric Type 1 Diabetes Mellitus

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Received March 22, 2010

Accepted September 10, 2010

On-line May 16, 2011

Summary

The aims of our study were to evaluate plasma levels of gut hormones in children with Type 1 diabetes mellitus (T1DM) in comparison with healthy controls and to correlate plasma concentrations of gut hormones with blood biochemistry, markers of metabolic control and with anthropometric parameters. We measured postprandial levels of specific gut peptide hormones in T1DM children. Amylin, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP-1), ghrelin, leptin, pancreatic polypeptide (PP), and polypeptide YY (PYY) were assessed in 19 T1DM children and 21 healthy reference controls. Multiplex assay kit (LINCOplex®) was used for determination of the defined plasma hormone levels. T1DM subjects had significantly reduced amylin ($p < 0.001$) and ghrelin ($p < 0.05$) levels, whereas GIP ($p < 0.05$) was elevated when compared with healthy controls. Plasma levels of other measured hormones did not differ statistically between the studied groups. Further analysis of T1DM patients demonstrated an association between body mass index and GLP-1 ($r = 0.4642$; $p < 0.05$), leptin ($r = 0.5151$; $p < 0.05$), and amylin ($r = 0.5193$; $p < 0.05$). Ghrelin levels positively correlated with serum HDL cholesterol ($r = 0.4760$; $p < 0.05$). An inverse correlation was demonstrated with triglycerides (TG) ($r = -0.5674$; $p < 0.01$), insulin dosage ($r = -0.5366$; $p < 0.05$), and HbA1c% ($r = -0.6864$; $p < 0.01$). Leptin was inversely correlated with TG ($r = -0.6351$; $p < 0.01$). Stepwise regression analysis was performed to enlighten the predictive variables. Our study demonstrated an altered secretion pattern of gut peptide hormones in T1DM children. A close correlation was revealed between these peptides as well as with blood biochemistry, markers of metabolic control and with

anthropometric parameters. Further studies are essential to explore this issue in T1DM children.

Key words

Diabetes mellitus type 1 • Gut peptide hormones • Children

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Introduction

Type 1 Diabetes mellitus (T1DM) is a chronic metabolic disorder in which autoimmune destruction of β -cells leads to lack of insulin impaired glucose metabolism (Adkinson *et al.* 1994). Hyperglycemia may increase oxidative stress with a subsequent impact on the entire organism. Our recent study supports increased oxidative stress markers in T1DM children (Varvarovska *et al.* 2004). Despite many efforts, the pharmacological treatment of T1DM consists of an unphysiological attempt to substitute only one of the hormones which are lost after β -cells destruction, namely insulin. Insulin replacement therapy does not mirror normal insulin secretory profiles, and persistent postprandial hyperglycemia is not uncommon in T1DM children. Reflective of this, many patients receiving intensive insulin therapy are unable to achieve the recommended

Table 1. Effects of gut peptides.

Effects of the specific GIT hormones								
GIT hormones	Hormone synthesis	GIT motility	Gastric emptying	Hepatic glucose output	Circulation levels		Insulin secretion	B-cells population maintain
					Fasting	Satiety		
<i>Amylin</i>	<i>pancreas</i>	↓	↓	↓	↓	↑	↑	-
<i>Ghrelin</i>	<i>GIT</i>			↑	↑	↓	↓	-
<i>GLP-1</i>	<i>GIT</i>	↓	↓	↓	↓	↑	↑	↑
<i>GIP</i>	<i>GIT</i>	↓	↓	↓	↓	↑	↑	↑
<i>Insulin</i>	<i>pancreas</i>	-	-	↓	↓	↑	N/A	-
<i>Leptin</i>	<i>addipose tissue</i>	-	-	↓			↑↓	-
<i>Pancreatic peptide</i>	<i>pancreas</i>	-	-	↓	↓	↑	↑	-
<i>PYY</i>	<i>GIT</i>	↓	↓	-	-	-	-	-

↑ - increased effect, ↓ - decreased effect, N/A - non-applicable, GIT – gastrointestinal tract.

Adapted from references: Kong *et al.* 1997, Panagiotidis 1992, Dezaki *et al.* 2008, Sun *et al.* 2008, Soriano-Guillen *et al.* 2004, Martos-Moreno *et al.* 2006, Celi *et al.* 2005, Zhang *et al.* 1994, Sinha *et al.* 1998, Polak *et al.* 1976, Kono *et al.* 2005, Brunicardi *et al.* 1996, Mannon *et al.* 1994, Spånge *et al.* 1998.

glycated hemoglobin A1c (HbA1c) target (Kieiss *et al.* 1998). Hormones closely related to glucose homeostasis are presented in Table 1.

Amylin

This 37-aminoacid hormone has important effects on glucose metabolism. Packed within insulin secretory vesicles, amylin is co-secreted by insulin by β -cells in response to feeding (Badman *et al.* 1996). Amylin affects insulin secretion and sensitivity, delays gastric emptying and inhibits glucagon secretion. This contributes to improving glycemia (Panagiotidis *et al.* 1992, Kong 1997). Recent evidence suggests that amylin is reduced in T1DM (Heptulla *et al.* 2008).

Incretins

Glucose-dependent insulintropic polypeptide (GIP) and glucagon-like peptide 1 (GLP-1), also called incretins, are intestinal hormones that are released in response to feeding. GIP is synthesized in enteroendocrine cells (K cells) located primarily in the proximal small intestine (Ross *et al.* 1978). GLP-1 is secreted with L cells that are scattered throughout the small bowel and ascending colon (Doyle *et al.* 2007). Circulating levels of GIP and GLP-1 are low in the fasted state and rise within minutes following food ingestion, returning to basal levels within 2-3 hours (Ranganath *et*

al. 1998). It has been reported that GIP and GLP-1 knockout mice revealed diabetes mellitus as the main consequence of the lack of incretin effect (Scrocchi *et al.* 1998). GIP achieves insulintropic effect by binding to a special receptor called GIPR (Yip *et al.* 2000). Lynn *et al.* (2001) reported that hyperglycemia alters the physiological response as a result of downregulation of GIPR expression (Lynn *et al.* 2001, Zhou *et al.* 2007). GLP-1 acts through GLP-1 receptors (GLP-1Rs) located in various tissues including the pancreas. Incretins effects are shown in Table 1. The role of GIP and GLP-1 has not yet been established in T1DM children.

Ghrelin

This is a 28-amino acid peptide predominantly produced by ghrelin cells in the antrum of the stomach (Rindi *et al.* 2004). Ghrelin plasma levels are reported to increase in anorexia and cachexia and reduced in obesity (Cummings *et al.* 2001). In normal subjects, ghrelin levels increase during fasting, and fall upon feeding (Broglia *et al.* 2005). Ghrelin has wide ranging actions such as controlling energy balance and endocrine actions. This includes regulation of growth hormone (GH) secretion and an inhibition of insulin secretion and an increase in blood glucose (Dezaki *et al.* 2008). Sun *et al.* (2008) reported that deletion of ghrelin not only improves β -cell function, but also increases peripheral tissue insulin

sensitivity (Sun *et al.* 2008). Low plasma levels of ghrelin and an impairment of ghrelin response to meals has been recently reported in T1DM (Celi *et al.* 2001, Soriano-Guillen *et al.* 2004, Martos-Moreno *et al.* 2006).

Leptin

Leptin is a 16-kD peptide which is mainly synthesized and released from adipocytes in white adipose tissue (Ranganathan *et al.* 1998), its expression has been also detected in a number of additional tissues such as the placenta, mammary epithelial cells and even in gastric mucosa (Mix *et al.* 2000). Leptin acts in the hypothalamus to suppress appetite and regulate body weight (Zhang *et al.* 1994). Meal ingestion does not acutely regulate serum leptin levels (Sinha *et al.* 1998). It has been hypothesized that leptin could act as a naturally occurring insulin sensitizer *via* a PI3-kinase-mediated pathway. This leads to both insulin-like effects on glucose transport and glycogen synthesis (Berti *et al.* 2007). However, at higher concentrations leptin impairs activation of insulin receptor autophosphorylation and phosphorylation (Pérez *et al.* 2004).

Pancreatic polypeptide (PP)

PP a 36-amino acid peptide is secreted by specialized pancreatic islet cells (PP cells). Its production is scattered throughout the exocrine parenchyma as well as being found in the periphery of the Langerhans islets (Adrian *et al.* 1976). Following a meal, pancreatic polypeptide is released into the blood from PP cells of the pancreas (Hazelwood *et al.* 1973, Polak *et al.* 1976). Kono *et al.* (2005) reported infusion with PP reduced insulin requirement in pancreatectomized dogs. PP infusions were also able to improve glycemic profiles in both in patients and animals with chronic pancreatitis (Brunicardi *et al.* 1996). T1DM adult patients appear to have elevated levels of pancreatic polypeptide (Tsuda *et al.* 1980). This suggests an association between PP levels and insulin sensitivity. The exact mechanism of action is under debate. Levels of PP have not been assessed in T1DM children.

Peptide YY (PYY)

L cells of the gastrointestinal tract (GIT) are a major source of PYY. In contrast to PP, PYY levels change very slowly and not to the same degree in response to a meal (Batterham *et al.* 2003). PYY inhibits gastric emptying and delays intestinal transit (Mannon *et al.* 1994). It has been reported that low levels of PYY

strongly correlated with decreased insulin sensitivity. In animal models of autoimmune T1DM the number of colonic PYY cells was reduced in diabetic, but not in pre-diabetic mice (Spånge *et al.* 1998). To our knowledge there are no studies assessing plasma levels of PYY in T1DM children.

Gut peptides have been studied in adults and animal models. However, there are still many controversial and unresolved issues regarding gut peptides in relation to T1DM in children. The goals of our study were to (1) evaluate plasma concentrations of amylin, ghrelin, GIP, GLP-1, leptin, insulin, PP, PYY in T1DM children and healthy controls; (2) to examine the possible interaction between the estimated hormone values; (3) to correlate gut peptides with blood biochemistry, markers of metabolic control and with anthropometric parameters.

Materials and Methods

Patients

Following approval by the institutional review board at the University teaching hospital in Pilsen, 40 eligible study participants were included in the study. A group of 19 T1DM patients were matched with a control group of 21 healthy children for sex, age and BMI (Body Mass Index). The characteristics of the studied groups are shown in Table 2. T1DM patients were recruited from the outpatient clinic of the Department of Endocrinology-Diabetology at the Charles University Teaching Hospital in Pilsen (Czech Republic). All data were collected within a 12-month period. Patients with other autoimmune diseases, liver disease, abdominal pain, infection, positive *Helicobacter pylori* infection status, renal dysfunction were excluded. None were using antibiotics, non-steroid anti-inflammatory drugs and medication known to affect GIT motility or appetite.

Study outline

Patients and matched controls fasted overnight at least 12 hours before a standardized breakfast was eaten (total energy: 1633 kJ, carbohydrates 38.7 g, fats 18.3 g, proteins 18.1 g, fiber 2.98 g). The breakfast corresponded to 3 diabetic exchange units. Only diabetic patients administered regular morning insulin dosages. Blood was drawn between 09.00 and 10.00 a.m. 90 minutes following the meal. The healthy controls (HC) did not apply artificial insulin otherwise the identical protocol was followed. Analysis included demographic data,

gender, height, weight, disease duration, hemoglobin A_{1c} (HbA_{1c} %), daily insulin dose, actual glycemia, and lipid profile. BMI for age was calculated as weight (kg) divided by the square of subject height in meters (m²).

Table 2. Demographic details and diabetic history.

Characteristics	T1DM	Controls
Subjects (n)	19	21
Sex (male/female)	12/7	10/11
Age (yr)	13.38 (3.25)	13.36 (3.50)
Height (cm)	150 (21.00)	153 (12.00)
Weight (kg)	46.08 (19.90)	45.77 (12.80)
BMI (kg/m ²)	19.49 (4.34)	19.06 (2.08)
Age at diagnosis (yr)	6.87 (2.47)	N/A
Duration of diagnosis (yr)	6.5 (2.22)	N/A
Insulin/kg/day (IU)	0.92 (0.28)	N/A
Glycemia (mmol/l)		
- before the meal	8.25 (3.38)	N/A
- following the meal (90 min)	12.50 (5.59)	5.28 (0.53)
Glycated hemoglobin A1C (%)		
- IFCC format	7.2 (0.28)	N/A

Number are expressed as mean values with standard deviation, *T1DM – Diabetes mellitus type 1 patient, *NS - Non-significant, *N/A - Non-available, * IFCC - International federation of clinical chemistry and laboratory medicine.

Analysis and laboratory tests

Multiplex assay kit (LINCOplex[®]) was used for determination of defined gut hormones levels (ghrelin, leptin, GIP, GLP-1, Amylin, PP, PYY, and insulin). Measurement accuracy was warranted with company standards concentrations. All samples were collected by the same qualified person. Blood samples were collected in ice-chilled EDTA-treated tubes. After collecting blood, LINCO's DPPIV inhibitor (for GLP-1 measurement), Sigma's Protease inhibitor cocktail (for Amylin measurement), and Roche's Pefabloc SC serine protease inhibitor (for active ghrelin measurement) were added. Plasma was separated by centrifugation (at 2200 PM, 12 min) and 3 samples of 200 µl were prepared and stored at -20 °C for later analysis. Thus, each patient's plasma sample was assayed 3 times within one session and the mean value was statistically analyzed. Samples did not undergo more than two freeze/thaw cycles. Intra-assay coefficient of variation (CV) was < 11 %, and inter-

assay < 19 %.

C-peptide was assessed using an immunoradiometric assay (Immunotech, Czech Republic, SR 300 Stratec[®] instrument). HbA_{1c} was measured by high performance liquid chromatography (Tosoh G7). Serum for high-density lipoproteins (HDL-C), low-density lipoproteins (LDL-C) and triglycerides (TG-C) were routinely measured using standard methodology (enzymatic assay GTP-PAP Human[®] and CHOD-PAP Dialab[®] respectively); all laboratory data was obtained through the hospital clinical laboratory.

Statistical analysis

Descriptive results are expressed as mean (standard deviation, S.D.) or median (interquartile range). Differences among groups were examined by non-parametric Wilcoxon's test. Categorical values are presented as relative frequencies examined by Fisher's exact test. Relationships among particular hormone concentrations in the T1DM patients with were analyzed using both Spearman's and Kendall's rank correlation. Further correlations among hormone concentrations and HbA_{1c} (mmol/mol), daily insulin dosage (IU/kg), actual glycemia, BMI (kg/m²) and lipid metabolism (HDL-C, LDL-C, and triglycerides) were analyzed using Spearman's and Kendall's rank correlation. Furthermore, statistically significant correlations were assessed by stepwise regression analysis. All statistical analyses were performed using Statistica[®] software (StatSoft, Tulsa, U.S.A.). A P-value of 0.05 was considered statistically significant.

Results

There were no significant differences regarding age, gender distribution, race/ethnicity distribution, height, weight and BMI between the study groups.

Gut hormones

Amylin

Plasma amylin levels of the children with T1DM were significantly lower when compared to healthy controls (HC) (50.11±27.30 pg/ml; median: 27.40 pg/ml, range: 27.30-113.31; 129±46.50 pg/ml; median: 129.93, range: 65.06-223.59; p<0.001) (Fig. 1).

GIP

Plasma GIP levels of the children with T1DM were significantly elevated GIP levels than in the HC

(234.21±157.29 pg/ml; median: 235.45 pg/ml, range: 49.15-561.07; 88.30±86.82 pg/ml (median: 53.70 range: 2.70-348.30; $p<0.001$) (Fig. 1).

GLP-1

A statistical difference between plasma levels of GLP-1 in T1DM patients and HC was not found (29.68±16.49 pg/ml; median: 28.54 pg/ml, range: 14.65-36.61; 23.44±13.51 pg/ml median: 17.75, range: 15.86-27.60; $p=0.39$) (Fig. 1).

Ghrelin

Plasma ghrelin levels in T1DM were significantly lower than in controls (41.20±27.91 pg/ml; median: 32.61 pg/ml, range: 13.70-97.53; 66.66±41.72 pg/ml; median: 61.76, range: 13.70-167.87; $p<0.05$) (Fig. 1).

Insulin

The plasma levels of artificially administrated insulin were significantly elevated among T1DM subjects compared to inherent insulin levels in HC subjects (1783.44±2896.72 pg/ml; median: 1110.58, range 137-13155.61; 323.53±119.12 pg/ml; median: 296.45; range 176.34-591.37; $p<0.001$).

C-peptide

C-peptide levels in T1DM patient were significantly decreased compared to those in HC subjects (50.45±96.00 pg/ml; median: 21.70, range: 3.62-43.40; 2072.36±717.93 pg/ml; median: 1945.95, range 1410.63-2336; $p<0.001$) (Fig. 1).

Leptin

A statistical difference between plasma levels of leptin in T1DM patients and HC was not found (4451.68±5698.27 pg/ml; median: 1857.60 pg/ml, range: 294.97-20814.95; 5682.77±6356.17; 2734.01 pg/ml, range: 607.51-22342.25; $p=0.71$) (Fig. 1).

PP

A statistical difference between plasma levels of PP in T1DM patients and HC was not found (139.00±96.38 pg/ml; median: 111.18 pg/ml, range: 29.73-351.71; 106.16±64.42 pg/ml; median: 74.96 pg/ml, range: 39.39-239.56; $p=0.21$) (Fig. 1).

PYY

A statistical difference between plasma levels of PYY in T1DM patients and healthy controls was not found (78.87±30.12 pg/ml; median: 71.24 pg/ml, range: 42.99-169.19; 84.75±29.71 pg/ml; median: 79.55 pg/ml, range: 60.39-104.54; $p=0.55$, Fig. 1).

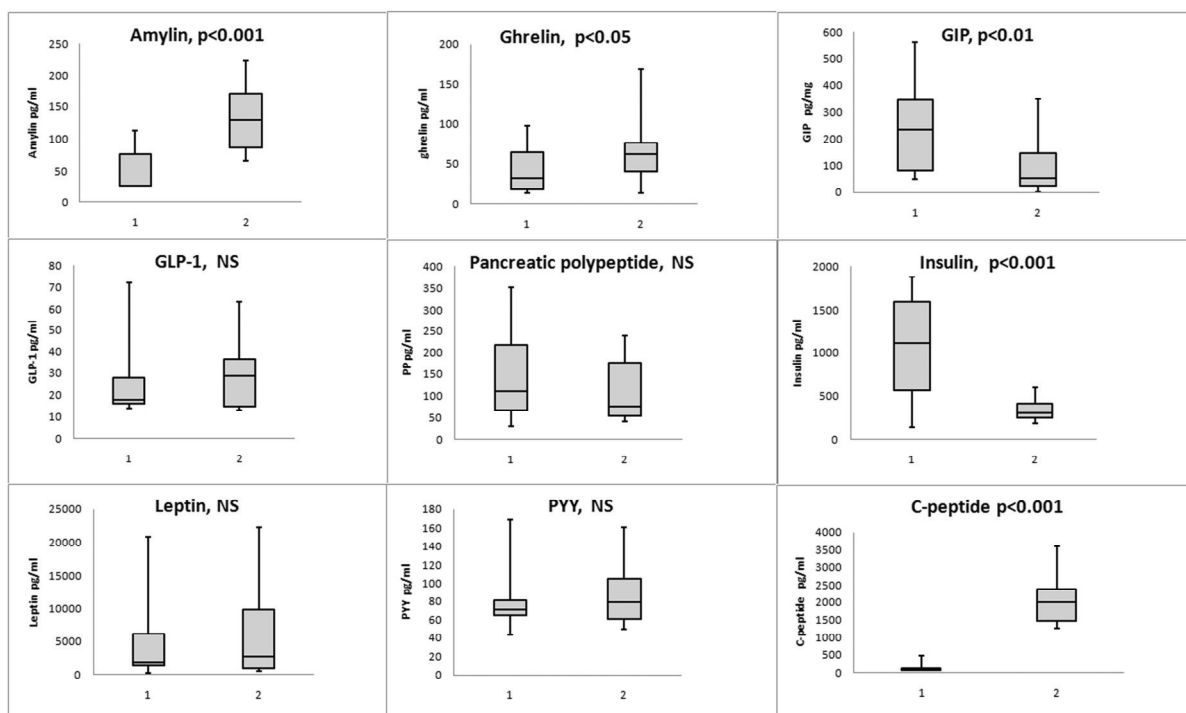


Fig. 1. Gut peptides. Boxes indicate the interquartile range. Horizontal lines within boxes indicate medians. Whiskers extend to the highest or lowest value. 1. Diabetes mellitus type 1 patients. 2. Healthy controls. NS - non-significant difference. p - p value.

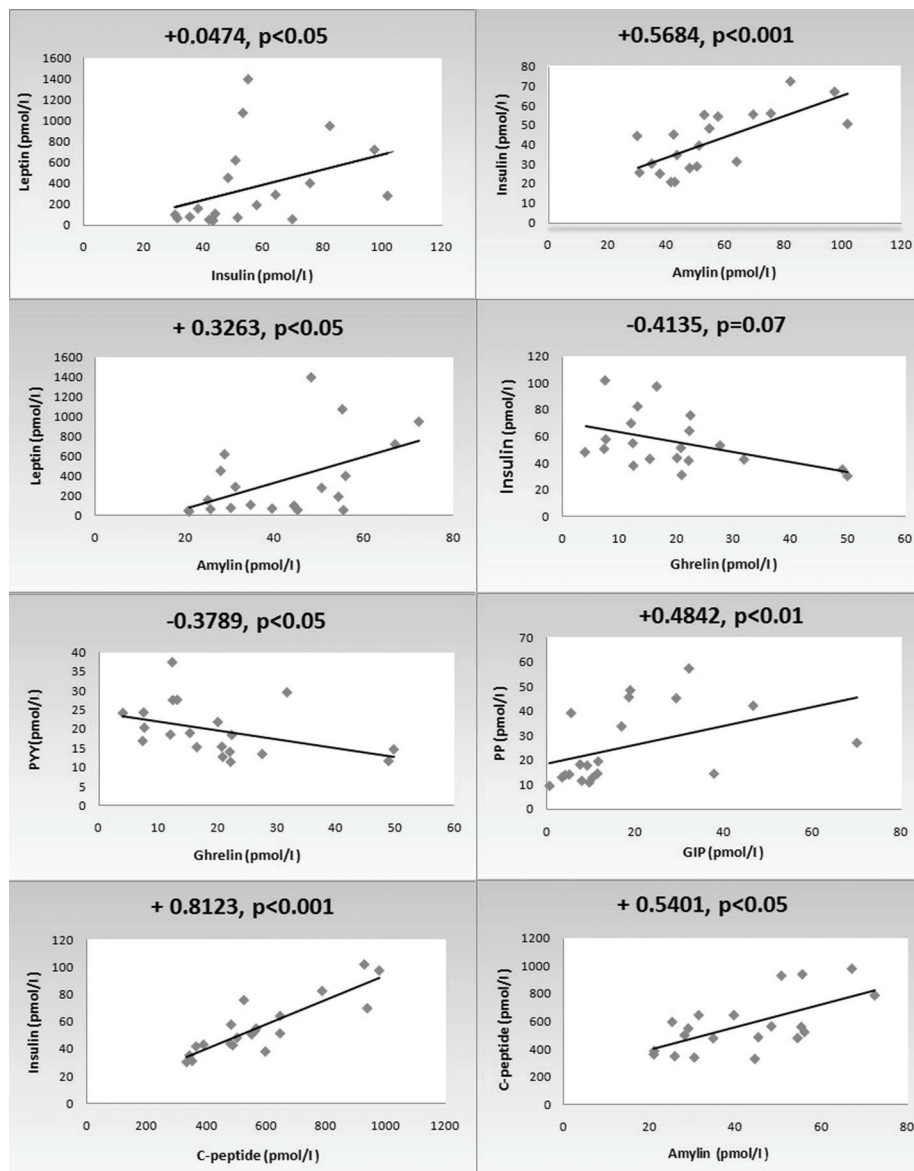


Fig. 2. Gut hormones correlations in the healthy controls. Relationships among particular hormone concentration in healthy controls. p - p value.

Correlation analyses

Correlation of plasma GIT peptides concentrations between studied groups

Healthy controls

Amylin levels correlated with insulin ($r=0.7474$; $p<0.001$), C-peptide ($r=0.5401$; $p<0.001$) and leptin ($r=0.4857$; $p<0.05$); insulin positively correlated with leptin ($r=0.5383$; $p<0.05$) and C-peptide ($r=0.8123$, $p<0.001$). Also proportional relationships were found between GIP and GLP-1 ($r=0.3810$, $p<0.05$), and PP ($r=0.6526$; $p<0.05$). In addition, there was an inverse correlation between ghrelin and PYY ($r= -0.5323$; $p<0.05$). (Fig. 2)

T1DM patients

Among the T1DM subjects, a significant proportional correlation was found between amylin and

GLP-1 ($r=0.7834$; $p<0.01$), and PP ($r=0.4867$; $p<0.05$). GIP correlated with GLP-1 ($r=0.4903$; $p<0.05$) and PP ($r=0.5702$; $p<0.05$). GLP-1 positively correlated with PP ($r=0.7223$; $p<0.01$) and PYY ($r=0.4569$; $p<0.05$), and insulin levels negatively correlated with plasma levels of PYY ($r= -0.4704$; $p<0.05$) (Fig. 3).

Correlation of plasma GIT peptide concentrations, blood biochemical characteristics and metabolic and clinical parameters of children with T1DM

Plasma levels of investigated hormones were correlated in the group of T1DM patient against patient's HbA1c, BMI, actual glycemia, serum concentration of TC, HDL-C, LDL-C, and TG, dosage of insulin per kilo (IU/D), age, sex, age at time of diagnosis, and years of T1DM duration. Patient's BMI was linked with GLP-1 ($r=0.4642$; $p<0.05$), leptin ($r=0.5151$; $p<0.05$), and

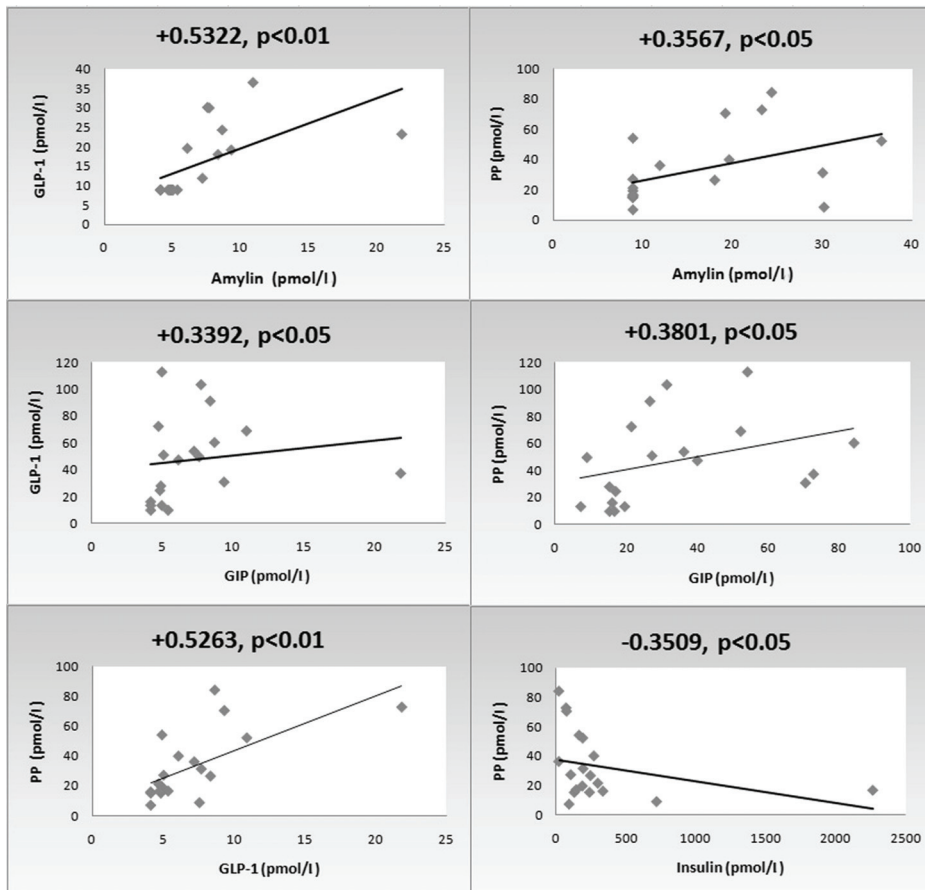


Fig. 3. Gut hormones correlations in Type 1 Diabetes mellitus patients. Relationships among particular hormone concentrations in Diabetes mellitus patients. p – p value.

amylin ($r=0.5193$; $p<0.05$). Ghrelin levels positively correlated with serum HDL ($r=0.4760$; $p<0.05$) and negatively with TG ($r= -0.5674$; $p<0.01$) and insulin dosage (insulin IU/kg/day) ($r= -0.5366$; $p< 0.05$). There were also inverse correlations between concentration of ghrelin and HbA1c ($r= -0.6864$; $p<0.01$), leptin and TG ($r= -0.6351$; $p< 0.01$). Insulin dosage (insulin IU/kg/day) was negatively associated with actual glycemia ($r= -0.3595$; $p< 0.05$) in T1DM. No other association between hormones and variables were found.

Stepwise analysis

Stepwise regression analysis revealed predictive variables for plasma ghrelin levels such as HbA1c%, ($r'=0.592$; $p<0.05$), HDL ($r'=0.790$; $p<0.001$) and insulin dosage ($r'=0.583$; $p<0.05$), and for plasma leptin levels such as BMI ($r'=0.818$; $p<0.001$), TG ($r'= -0.793$; $p<0.001$) and cholesterol ($r'= -0.527$; $p<0.05$). No other association between hormones and variables were found.

Discussion

In the present cross-sectional study, we sought to determine the circulating levels of seven gut hormones in

T1DM children. To our knowledge, this is the first study providing information regarding 7 gut hormones in T1DM children. The T1DM children and adolescents in our study sample displayed significant differences in plasma concentrations of the analyzed hormones when compared with healthy controls. The low levels of stimulated C-peptide in our T1DM subjects reflect a severe depletion of their insulin reserves (The diabetes control and complications trial research group 1998). We suggest that an assessment of gut hormone secretion profiles without distortion of preserved β -cells function.

On the basis of this analysis we demonstrated decreased amylin levels in T1DM children ($p<0.001$). Our research concurs with data provided by studies with adult subjects (Heptulla *et al.* 2005). T1DM individuals lack important amylin effects during the postprandial period – suppression of glucagon secretion by pancreatic α -cells and delaying gastric emptying (Panagiotidis *et al.* 1992, Kong 1997). Given that it may be beneficial to restore amylin deficiency in T1DM patients. Our study revealed that amylin levels positively correlated with GLP-1 and PP levels in T1DM subjects, but not in

controls. Low GLP-1 levels are associated with diabetic states as well as changes in circulating PP levels impact insulin sensitivity (Tsuda *et al.* 1980, Greenbaum *et al.* 2002). Amylin levels also positively correlate with C-peptide levels in healthy controls. Significantly low levels of both peptides have been assessed in T1DM suggesting declined secretion of their β -cells. A synthetic amylin receptor agonist has been developed and approved for the treatment of T1DM adult patients in combination with insulin (Edelman *et al.* 2008). In long-term therapy amylin agent has been found to reduce postprandial glucose excursions, lower HbA1c and weight in subjects with T1DM – without a concomitant increase in the insulin dose (Whitehouse *et al.* 2002, Ratner *et al.* 2004, Hassan *et al.* 2009). Another double-blind, placebo controlled trial also demonstrated a reduction in postprandial glucose excursions, weight in subjects, whereas no significant difference was found in HbA1c (Edelman *et al.* 2006). Given the current findings, a potential use of amylin to reduce postprandial hyperglycemia can be envisioned as a new element of T1DM treatment in children.

The roles of GIP and GLP-1 have not been fully explored in T1DM children yet. Previous studies have reported normal circulating levels of GIP in newly diagnosed T1DM young adults (Krarup *et al.* 1985, 1988). Nevertheless, our T1DM patients showed elevated levels of GIP when compared to healthy controls. The different findings in our study may be partly explained by the duration of diabetes in our subjects. Our T1DM patients were diabetics with more than 6.5 years disease duration with limited insulin secretion based on C-peptide levels, whereas two previous above mentioned studies examined newly diagnosed cohorts (Krarup *et al.* 1985, 1988). It is well documented that once insulin treatment is commenced; most likely the β -cell function improves for a certain period of time. In spite of continuous β -cell destruction in T1DM states, some of these cells are still present for a long period of time from initial diagnosis (Couri *et al.* 2008). It could be speculated that these elevated GIP levels may occur as a compensatory mechanism for the low number of β -cells along with either chronic desensitization of GIPRs or a reduction in the expression of GIPRs on pancreatic β -cells in T1DM children. Furthermore we observed that GIP is also positively correlated with GLP-1 and PP in both T1DM patients and the controls. These correlations could be attributed to counter-regulatory mechanisms in relation to impaired glucose homeostasis. However, the

mechanism by which GIP influences GLP-1 and PP is not fully understood. Given that any pharmacological interference in GIP pathway would be limited as target cells are in low numbers and perhaps express insufficient numbers of GIPR. These findings warrant further confirmation.

We found lower plasma GLP-1 concentrations in T1DM children when compared to controls, although there was no statistical significance. Greenbaum *et al.* (2002) reported the lower incretin effect in T1DM subjects by matching glucose values between the intravenous and oral administration, but incretin levels did not differ when compared to controls (Greenbaum *et al.* 2002). Interestingly, fasting hyperglycemia was significantly improved in a group of T1DM subjects with minimal insulin secretory reserve, who were given GLP-1 agents. This was most likely due to suppressed glucagon secretion (Creutzfeldt *et al.* 2007). According to our results, GLP-1 is associated with PP and PYY levels ($p < 0.01$, $p < 0.05$ respectively) in T1DM. Altered PP levels have been reported in association with T1DM (El-Salhy *et al.* 2001, Tsuda *et al.* 2001). In our study, levels of GLP-1 also positively correlated with patient's BMI ($p < 0.05$). A recent study described an association between GLP-1 and BMI (De Luis *et al.* 2007). This might imply that increased GLP-1 levels act as a counter mechanism preventing further caloric intake and weight gain. Thus perhaps GLP-1 can be envisioned as more useful than GIP as a new potential target in treating T1DM.

There is limited research on the role of ghrelin, an appetite-enhancing hormone, in T1DM. Few studies have reported ghrelin concentrations in T1DM patients, and with controversial results (Celi *et al.* 2001, Soriano-Guillen *et al.* 2004, Martos-Moreno *et al.* 2006). Holdstock *et al.* (2004) reported altered ghrelin secretion in T1DM children with disease onset which returned to normal after 9 months of insulin treatment. Our T1DM patients had lower postprandial active ghrelin concentrations ($p < 0.05$) which is in agreement with the majority of previous studies of T1DM children. As previously described active ghrelin deficiency augments insulin secretions in response to glucose load (Dezaki *et al.* 2008). Of note, we found a significant negative correlation between ghrelin and insulin dosage among the T1DM subjects and a borderline correlation between ghrelin and insulin among the healthy controls. In a study of adults with T1DM, a negative correlation was found between ghrelin and insulin dosage (Celi *et al.* 2005).

Based on our data and available literature, we can hypothesize that an excess insulin administration among T1DM patients needed to surmount long-term hyperglycemia leads to suppressed ghrelin levels which, in turn, increases peripheral tissue insulin sensitivity and improves function of remaining β -cells. Furthermore we also found a negative correlation between ghrelin and HbA_{1c} ($p < 0.01$), which was identified together with insulin dosage and HDL levels as a predictive factor. Based on our findings we could speculate that postprandial ghrelin values might serve as a parameter of metabolic compensation in the future as a significant correlation exists with HbA_{1c}. The positive correlation between ghrelin and HDL and inverse relationship with TG levels ($p < 0.05$, $p < 0.01$ respectively) supports ghrelin-insulin linkage. As well-controlled T1DM patients have normal HDL levels. Hypothetically, insulin excess present in patients with either T1DM or type 2 diabetes mellitus may play an important pathophysiological role in accelerating atherogenic processes by reducing the beneficial effect of HDL cholesterol (Brazg *et al.* 1993). Nevertheless, these analyses should be interpreted with caution. Replication of these findings in a larger sample is required.

Our results have indicated that the levels of leptin were normal in our T1DM patients and showed no statistical differences compared with healthy controls. Kiess *et al.* (1998) reported low leptin levels in T1DM children before the treatment with insulin, but normal levels in pre- and early pubertal patients on insulin therapy. Elevated leptin levels were found in late and post-pubertal T1DM patients. We assumed that the difference between these studies could be attributed to patient group distribution. Our study did not contain enough pubertal subjects for adequate statistical analysis; though our findings provide evidence that leptin may play an important role in insulin metabolism. We also found a positive correlation between leptin and amylin and insulin in healthy controls ($p < 0.05$, $p < 0.05$ respectively). In our T1DM group, leptin levels positively reflect BMI ($p < 0.05$). BMI, TG and cholesterol were also identified as predictive factors for leptin levels. One of the well-known side effects of intensified insulin treatment of T1DM adolescent is weight gain which leads to higher leptin levels. This suggests that insulin administration may contribute to the synthesis of leptin. It has been reported that high leptin levels are associated with insulin insensitivity (Kiess *et al.* 1998). It is questionable whether or not high leptin levels are protective of the organism by

decreasing insulin sensitivity against further weight gain. This also raises the question whether higher leptin levels related to pubertal status are associated with higher daily insulin dosages.

There are no clinical reports available on PP levels in T1DM children in the international literature. However, it is documented that there is hyperplasia of PP cells after DM onset in young adult diabetics (Orci *et al.* 1976). Elevated fasting and postprandial levels of PP have been reported in young T1DM adults (Tsuda *et al.* 1980). Our group of T1DM subjects tended to have higher PP levels although this was not statistically significant. Elevated PP levels might be attributed to hyperglycemia in T1DM states as PP increases liver sensitivity to insulin and reduces the amount of glucose produced by the liver (Brunicardi *et al.* 1996, Kono *et al.* 2005). With fewer excursions in blood glucose, a patient should decrease the amount of insulin required. The PP effects support a positive relationship with amylin ($p < 0.05$) and a negative correlation between PP and insulin ($p < 0.05$) in T1DM demonstrated in our study. We presume that PP agents should draw more attention as a potential candidate of treating agents in T1DM children.

The role of PYY in T1DM children has not been extensively studied in recent years. In concordance to other studies using animal models, we observed a trend of decreased PYY. As PYY inhibits gastric emptying, the number of PYY cells and PYY synthesis are decreased in an attempt to compensate slow gastric emptying during hyperglycemia (Spånge *et al.* 1998). On the other side PYY cells have been investigated in the rectum of patients with long duration T1DM (13-48 years) with gastrointestinal symptoms (nausea, diarrhea) (El-Salhy *et al.* 2001). Contrary to animal models the number of rectal PYY cells in these patients has been found to be significantly higher than that of healthy volunteers. It is tempting to speculate that the changes in PYY are dynamic and change with the course of the diabetic state, also regarding insulin therapy. More detailed studies should be undertaken to measure PYY levels at different stages of diabetes or at the presence of motility disturbance in T1DM. In addition, a trend towards an inverse correlation was observed for ghrelin and PYY and a positive correlation between GLP-1 and PYY was found. This relationship might support the theory of insufficient incretin effect and a central role for GLP-1. PYY levels might be normalized by administering GLP-1 as well as bowel transit and glucose profiles. Studies on larger cohorts are required.

Limitations should be considered in the interpretation of our findings. The study design was intended to produce results that account for or predict the relationship between T1DM and gut hormones in children. Although the number of subjects is sufficient for statistical analysis, further studies with larger populations with longitudinal follow-ups are needed. Differences in insulin levels should be also considered as we have not found any studies investigating exogenous insulin and its effect on gut hormones levels in T1DM. Gut hormone levels could be assessed at several intervals following meal ingestion to produce a dynamic curve. In our study however, repeated blood analysis in children, even for assessing this valuable information, was neither feasible nor ethical.

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Conclusions

Our study demonstrated altered secretion patterns of gut hormones in T1DM children. The differences in gut hormones levels may impact on daily insulin dosages and metabolic control. Gut hormones are a promising future prospect for a deeper understanding of diabetic metabolism. Given our final data we believe that better metabolic control and lower morbidity could be achieved by pharmacological intervention in gut hormone pathways. Further studies are essential to explore this issue in T1DM children.

Conflict of Interest

There is no conflict of interest.

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