Reprint from The Review of DIABETIC STUDIES Vol 13 No 1 2016

The Review of DIABETIC STUDIES

ORIGINAL DATA

Esophageal and Gastric Dysmotilities are Associated with Altered Glucose Homeostasis and Plasma Levels of Incretins and Leptin

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Manuscript submitted June 16, 2015; resubmitted August 14, 2015; accepted August 29, 2015

■ Abstract

BACKGROUND: Gastrointestinal complications in diabetes may affect glucose and endocrine homeostasis. Glucose-dependent insulinotropic peptide (GIP), glucagon-like peptide-1 (GLP-1), and leptin regulate glucose homeostasis, food intake, and gastric emptying. **AIM**: The aim was to investigate associations between diabetes complications and glucose homeostasis and plasma levels of GIP, GLP-1, and leptin. **METHODS**: Sixteen diabetes patients (seven men) were examined with gastric emptying scintigraphy and 72-h continuous subcutaneous glucose monitoring, 14 with the deep-breathing test, and 12 with esophageal manometry. A fiber-rich breakfast was given during the second day of glucose registration. Blood samples were taken 10 min and right before a fat-rich breakfast, as well as 10, 20, 30, 45, 60, 90, 120, 150, and 180 min afterwards. 20 healthy volunteers acted as controls. Plasma was analyzed regarding GIP, GLP-1, and leptin by Luminex. **RESULTS**: Gastroparesis lowered maximal concentration (c-max) ($p = 0.003$) and total area under the curve (tAUC) ($p = 0.019$) of glucose levels as well as d-min ($p = 0.043$) of leptin levels. It tended to lower baseline ($p = 0.073$), c-max ($p = 0.066$), change from baseline (d-max) ($p = 0.073$), and tAUC ($p = 0.093$) of GLP-1 concentrations. Esophageal dysmotility tended to lower tAUC of glucose levels ($p = 0.063$), and c-min ($p = 0.065$) and tAUC ($p = 0.063$) of leptin levels. Diabetes patients had a higher baseline concentration of glucose ($p = 0.013$), GIP ($p = 0.023$), and leptin ($p = 0.019$) compared with healthy subjects. **CONCLUSIONS**: Gastric and esophageal dysmotility are associated with both lesser increases in postprandial glucose elevations and decreased postprandial changes in GLP-1 and leptin.

Keywords: autonomic neuropathy · diabetes mellitus · esophageal dysmotility · gastroparesis · incretins · leptin

1. Introduction

Gastroparesis is defined as delayed gastric emptying in the absence of any mechanical obstruction, and is thought to be caused by neuropathy, myopathy, and/or disturbances in interstitial cells of Cajal. Diabetes mellitus accounts for one-third of identified cases, but other metabolic or endocrine imbalances such as hypothyroidism, hyperglycemia, and hypokalemia may also cause gastroparesis [1].

The rate of gastric emptying results primarily from a negative feedback mechanism arising in response to nutrients in the small intestine, and is mediated by neuronal and hormonal mechanisms. Fat generates the most potent feedback because of its high caloric density [2]. Apart from gastroparesis, esophageal dysmotility is found in up to 60% of patients with diabetes [3]. Autonomic neuropathy is well established as a complication in diabetes, and is one possible etiology of gastrointestinal dysmotility [1, 4]. Both esophageal and gastric

dysmotility may affect postprandial glucose levels in diabetes [5].

Approximately 50% of the rise in plasma insulin after oral glucose load is due to the incretins glucose-dependent insulinotropic peptide (GIP) and glucagon-like peptide-1 (GLP-1), both of which are released within the first postprandial hour [6, 7, 8]. GIP is synthesized by K-cells of the duodenum and small intestine [6]. In man, GIP does not regulate gastric emptying [9], although a mouse study has suggested GIP to slow down gastrointestinal transit time [10].

GLP-1 is secreted by L-cells in the small intestine, primarily in the ileum [6]. GLP-1 has been shown to slow down gastric emptying after meals through vagal pathways; this mechanism contributes to metabolic control [11]. The effect of GLP-1 on gastric emptying takes place within minutes after food intake, and therefore appears to override insulin-dependent glucose control since insulin release takes place 30-40 min after food intake [7, 11, 12].

Leptin is secreted predominantly by adipocytes, resulting in a close relationship between plasma leptin levels and total amount of body fat [13]. Leptin is secreted in an exocrine fashion by ventricular chief cells in combination with pepsinogen, providing short-term regulation of food intake [14]. Luminal leptin stimulates the secretion of cholecystokinin (CCK), which in turn stimulates further leptin release [15]. In the periphery, leptin decreases the gastric emptying rate through CCK release followed by increased vagal afferent activities. Centrally, this effect is initiated by a direct action at the hypothalamus level [16]. Furthermore, leptin regulates the absorption of nutrients, e.g. increased peptide absorption [17], and is thought to be involved in the development of diabetes and obesity [18].

Abbreviations:

BMI	body mass index
CCK	cholecystokinin
CGMS	continuous glucose monitoring system
c-max	maximal concentration
c-min	minimal concentration
dAUC	delta area under the curve
d-max	maximal delta concentration
d-min	minimal delta concentration
E/I	expiratory/inspiratory ratio
E%	energy percentage
GIP	glucose-dependent insulinotropic peptide
GLP-1	glucagon-like peptide-1
HbA1c	glycosylated hemoglobin
IQR	interquartile range
LES	lower esophageal sphincter
MFI	median fluorescence intensity
PMT	photomultiplier tube
SD	standard deviation
tAUC	total area under the curve

Patients with type 2 diabetes have an impaired capability to regulate the incretin effect in response to increasing oral glucose loads, and thus, their insulinotropic effect is partly lost [7]. The reduced incretin effect is mainly caused by a reduced insulinotropic effect of GIP on pancreatic GIP receptors, while the effect of GLP-1 is well maintained [6]. In general, fat is a major determinant of the postprandial responses of gut peptides. Accordingly, studies have shown that both carbohydrate and fat content are of importance for the postprandial response of GLP-1 and GIP in healthy subjects and in patients with diabetes type 2 [19, 20, 21]. The effects of type 1 diabetes on incretin secretion or its effects after a fat-rich meal have been less well studied.

Previous studies have described how gastrointestinal dysmotility affects gut hormone release:

- Gastroparesis was associated with lower oxytocin levels.
- Esophageal dysmotility was associated with hyperCCKemia.
- Autonomic neuropathy was associated with hypergastrinemia [22].

However, the influence of gastrointestinal dysmotility or autonomic neuropathy on incretin or leptin secretion has not been fully elucidated in diabetes [6, 7, 15, 20, 21], although these hormones have been shown to affect gastrointestinal motility, and are influenced by idiopathic gastroparesis [8, 11, 16].

The aim of the present study was to examine the link between esophageal dysmotility and gastric dysmotility, and whether autonomic neuropathy, postprandial glucose homeostasis, and plasma levels of GIP, GLP-1, and leptin are associated events in a well-characterized diabetes cohort, and to compare basal plasma levels of these hormones in diabetes and health.

2. Material and methods

The study was performed according to the Declaration of Helsinki, and approved by the Ethics Committee of Lund University, for the patient cohort (LU 145-02), and for the healthy volunteers (2014/460). All patients and volunteers gave their written, informed consent before entering the study.

2.1 Diabetes subjects

Consecutive patients presenting with symptoms of gastroparesis at the Department of Endocrinology at Skåne University Hospital, Malmö, were invited to participate in the study. Patients with severe kidney or liver diseases, or regular use of opiates and other drugs influencing gastrointestinal motility, were excluded. Patients treated with prokinetic drugs had to withdraw the drugs 7 days before the examination. Patients with severe retinopathy were excluded as some examinations demanded good eyesight.

At the time of inclusion, the patients completed an established questionnaire regarding symptoms related to complications of the gastrointestinal tract, namely, loss of appetite, swallow complications, meal-related cough, early satiety, nausea, vomiting, weight loss, abdominal fullness, bloating, regurgitation, constipation, diarrhea, evacuation incontinence, symptomatic postprandial hypoglycemia, and postprandial perspiration [3, 5]. Patients were weighed and measured to calculate body mass index (BMI). Diabetes type, disease duration, and drug treatments were noted at inclusion. Diabetic complications examined included:

- Retinopathy based on fundus photography
- Angiopathy
- Microalbuminuria measured as albumin/creatinine ratio
- Albuminuria
- Peripheral neuropathy examined by patella and Achilles tendon reflexes, vibration sense, and monofilament
- Autonomic neuropathy according to established criteria (sexual dysfunction, profound sweating, and orthostatic blood pressure)

Glycosylated hemoglobin (HbA1c) was analyzed at the Department of Chemistry according to routine clinical practice. HbA1c values were collected as Mono-S (%), and subsequently converted to the International Federation of Clinical Chemistry (IFCC, mmol/mol) standard by use of the following algorithm: 10.45 x HbA1c (Mono-S) - 10.62 = HbA1c (IFCC) [23].

2.2 Basal examinations of diabetes patients

Gastric emptying scintigraphy was performed using a solid test meal, containing a technetium-99-labelled egg. Gastric emptying half-time (T_{50}) >2 standard deviation (SD) of healthy controls (= 70 min) was considered abnormal [24].

Esophageal manometry was performed with the patient sitting in an upright position, and each patient performing 10 swallows. The lower esophageal sphincter was identified during a slow pull-through in the mid-expiratory phase. The patient was considered to have esophageal dysmotility if one or more of the following criteria were fulfilled:

1. Absence of peristaltic contraction in the esophagus.
2. Hypo/hypercontractions, with a mean peristaltic amplitude <30 or >200 mmHg in the esophagus.
3. Percentage of simultaneous, non-propulsive peristaltic waves in the esophagus following a swallow >10%.
4. Speed of the peristaltic wave <3 or >6 cm/sec in the distal esophagus.
5. Resting pressure in the lower esophageal sphincter (LES) <10 or >30 mmHg [25].

The deep-breathing test was used to determine autonomic nerve function. The expiratory/inspiratory (E/I) ratio was calculated from the mean value of the longest R-R interval during expiration and the shortest R-R interval during inspiration. An age-related E/I ratio below -1.64 SD was considered abnormal [26].

Subcutaneous glucose levels were monitored during a 72-h period, using a continuous glucose monitoring system (CGMS) (Mini Med, Sylmar, CA, USA) and measured in mmol/l [27].

2.3 Healthy subjects: selection and definition

Healthy volunteers were recruited from the staff of the hospital or their friends and relatives; they constituted the control group. Subjects with no symptoms and no history of severe gastrointestinal disease, abdominal surgery, or diabetes mellitus were included. The subjects had no food allergy, or liver, renal, heart, pulmonary, cardiovascular, malignant, or psychiatric diseases, and no one was taking regular medication.

2.4 Subject characteristics

Of the 20 diabetes patients selected initially [5], only 16 were actually included in the study (seven men and nine women; median and interquartile range (IQR) of age (year): 54.0 (44.5-58.0); BMI (kg/m^2): 27.6 (20.5-29.4)). Four patients were excluded due to missing blood samples. Of the included patients, three had type 2 diabetes. All patients except one were treated with insulin.

All the 16 patients were investigated with gastric emptying scintigraphy and a continuous subcutaneous glucose monitoring over 72 h. Fourteen patients were examined with the deep-breathing test, and 12 with esophageal manometry. Of the 16 patients, 7 had gastroparesis; 7 of the 14 patients examined with the deep-breathing test had autonomic neuropathy, and 6 of the 12 patients examined with esophageal manometry showed esophageal dysmotility. Among the patients examined with the deep-breathing test, symptoms of autonomic neuropathy were present only in those who had a pathologic test result.

The most common symptoms in the patients were abdominal fullness (n = 14), bloating (n = 14), early satiety (n = 12), nausea (n = 10), and regurgitation (n = 9). No symptom was associated with objective signs of dysmotility (data not shown). Apart from gastrointestinal dysmotility and autonomy neuropathy, the patients also had retinopathy (n = 12), peripheral neuropathy (n = 11), nephropathy (n = 7), and angiopathy (n = 2).

Twenty healthy volunteers (8 men and 12 women; median (IQR) age (year): 48.0 (31.0-57.3); BMI (kg/m^2): 25.0 (22.2-26.9)) were included. Except for one healthy volunteer who chewed tobacco, all subjects were non-smokers and did not use snuff.

2.5 Study design

In the morning of the second day of subcutaneous glucose monitoring, the diabetes patients received a fiber-rich breakfast after an overnight fast and after taking their usual insulin injection. Glucose levels were extracted from the subcutaneous registration at 10 min and right before the meal, as well as 10, 20, 30, 45, 60, 90, 120, 150, and 180 min after the meal. The levels were used for statistical calculations.

On another day, a fat-rich test breakfast was given after an overnight fast. Blood samples were taken through an intravenous catheter 10 min and right before the meal, as well as 10, 20, 30, 45, 60, 90, 120, 150, and 180 min after the meal. Blood from healthy subjects was taken after 10 h of fasting. All samples consisted of whole blood drained into ethylenediaminetetraacetic acid (EDTA) glass tubes (BD Microtainer, New Jersey, USA). Measurement of glucose was performed from whole blood by HemoCue© (HemoCue AB, Ängelholm, Sweden) in healthy subjects, and before breakfast and 90 min after breakfast in diabetes patients. Subsequently, blood was immediately cooled and centrifuged at 3000 rcf for 5 min. Plasma was harvested and stored at -20°C until analysis of peptide concentration using Luminex.

2.6 Test meals

The fiber-rich test breakfast during CGMS consisted of oatmeal porridge, cooked from 35 g fiber porridge oats, 150 g milk (0.1% fat), 17 g mashed apple, a slice of rye bread containing 9% fiber, 5 g margarine (40% fat), and 10 g cheese (17% fat). The nutritional composition represented 350 kcal, 16.2 g (19 E%) protein, 7.4 g (18.8 E%) fat, and 49.6 g (57.3 E%) carbohydrate.

The fat-rich breakfast given prior to plasma peptide measurements contained 150 g cream and 150 g water. This meal amounted to 60 g fat and 561 kcal. It corresponded to the same fat content as used earlier to evoke CCK and oxytocin secretion [28].

2.7 Plasma analyses

Blood samples from patients with diabetes were analyzed by a human 5-plex panel (Bio-Plex Pro™ human diabetes immunoassay control numbers 5035092 and 5040782, Bio-Rad Laboratories, CA, USA). The analysis was performed on the Luminex-200 diagnostic system (Luminex xMAP, Bio-Rad Laboratories). The data were analyzed using Bio-Plex Manager software 6.0 (Bio-Rad). The following peptides were measured in pg/ml:

- Ghrelin
- GIP
- GLP-1
- Insulin
- Leptin

Blood samples from healthy volunteers were analyzed by a human diabetes 10-plex panel (Bio-Plex Pro™ Human Diabetes Immunoassay control numbers 5029560-1 and 5040782, Bio-Rad Laboratories, CA, USA). The analysis was performed on the Luminex-200 system (Luminex xMAP, Bio-Rad Laboratories). The data were analyzed using Bio-Plex Manager software 6.0 (Bio-Rad Laboratories). The following peptides were measured in pg/ml:

- C-peptide
- Ghrelin
- Glucagon
- GLP-1
- GIP
- Insulin
- Leptin

- Plasminogen activator inhibitor-1 (PAI-1)
- Resistin
- Visfatin

The analyses were performed according to the manufacturer's instructions. The acquired samples were diluted 1:4, and incubated with magnetic microspheres (beads) covalently coupled with capture antibodies directed against the desired biomarker. Thus, the coupled beads reacted with the sample containing the biomarker of interest.

Series of washes were performed using a magnetic wash station (Bio-Plex Handled magnetic washer, 171020100, Bio-Rad Laboratories) to remove any unbound protein from the samples. Then, biotinylated detection antibodies were added to form a sandwich complex. Finally, streptavidin-phycoerythrin (SA-PE) conjugates were added to the samples, with phycoerythrin serving as a fluorescent indicator (reporter) to form the final detection complex. Data from the reactions were acquired using the Luminex-200 system, which illuminated the fluorescent dyes within each bead (using a red laser, 635 nm) to provide bead classification, and thus, assay identification. Simultaneously, a green laser (532 nm) excites the phycoerythrin to generate a reporter signal, which is detected by a photomultiplier tube (PMT).

Absolute concentrations were measured from standards provided with the kit. Each run included controls, high and low concentrations of each biomarker, and blank. All samples were analyzed in duplicate, and the concentration of peptides bound to each bead was proportional to the median fluorescence intensity (MFI) of the reporter signal. Standard curves were calculated with type 5 parameter logistic regression. Inter- and intra-assay coefficients of variation (cv) for controls were:

- 8 (8.3%) and 8 (9.9%) for GIP
- 8 (10.5%) and 8 (13.9%) for GLP-1
- 8 (8.7%) and 8 (6.7%) for leptin

2.8 Statistical analysis

The statistical analysis was performed using SPSS (Statistical Package for the Social Sciences), version 22. The results were expressed as median (IQR). The baseline values were the mean of the two samples taken before the meal in the diabetes patient group, and from one sample in the control group. Maximum and minimum concentrations (c-max and c-min, respectively) were the highest and lowest concentrations measured in plasma or subcutaneously after the meal. The change from baseline (delta) is defined as d-max and d-min, respectively. In cases with two identical peak values in the same subject, the first one was used.

The area under the curve (AUC) was calculated for glucose, GIP, GLP-1, and leptin, and defined as total AUC (tAUC) and change in AUC from baseline (dAUC). There was no difference in any glucose or peptide levels between type 1 and type 2 diabetes. Therefore, calculations were carried out with the two groups combined. Since all but one of the diabetes patients were treated with insulin, and the ghrelin values were not reliable, these measurements were omitted from the calculation. In cases where one consecutive analysis was missing, a linear mean was calculated based on the values before and after the missing value. In cases where more than one consecutive analysis was missing, the subject was omitted from AUC calculations.

As several peptide analyses differed depending on gender among the diabetes patients, and the prevalence of gender differences varied between the diabetes subgroups, the values were gender-standardized using a linear regression model which considered gender as a covariate, and which expressed the variables as z-scores. Due to the limited size of both groups and an uneven distribution of values, the Mann-Whitney U-test was used for calculations to examine differences between groups. Fisher's exact test was used for dichotomous variables. Spearman's correlation test was used to study associations between glucose levels and peptide levels. $P \leq 0.05$ was considered statistically significant.

3. Results

3.1 Subject characteristics

There was no significant difference in age, BMI, duration of diabetes, and HbA1c levels between the groups with normal and abnormal gastric emptying (**Table 1**), esophageal motility (**Table 2**), and autonomous nervous system (**Table 3**). A difference was seen in the distribution of sexes between the groups related to gastric emptying and between patients with or without autonomic neuropathy.

3.2 Gut peptide levels in patients with diabetes

Basal and postprandial concentrations of glucose and peptides in the whole diabetes group are shown in **Table 4**. The baseline concentration of

Table 1. Characteristics of patients with and without gastroparesis

Characteristic	Delayed gastric emptying (n = 7)	Normal gastric emptying (n = 9)	p
Women	6 (85.7%)	3 (33.3%)	0.042
Age (yr)	49.0 (29.0; 55.0)	58.0 (51.5; 60.0)	0.055
BMI (kg/m^2)	28.0 (18.0; 30.0)	26.3 (21.2; 29.3)	0.908
Duration of diabetes (yr)	24.0 (13.5; 37.5)	33.0 (23.3; 46.8)	0.143
HbA1c (mmol/mol)	63.0 (40.0; 69.0)	69.0 (58.5; 77.0)	0.427
Esophageal dysmotility	5 (71.4%)	1 (11.1%)	0.093
Autonomic neuropathy	3 (42.9%)	4 (44.4%)	0.606

Legend: All data are given as median (interquartile range) or number and percentage, using Fisher's exact test and Mann-Whitney U-test. P ≤ 0.05 was considered statistically significant. *Abbreviations*: BMI – body mass index, HbA1c – glycated hemoglobin.

Table 2. Characteristics of patients with and without esophageal dysmotility

Characteristic	Esophageal dysmotility (n = 6)	No esophageal dysmotility (n = 6)	p
Women	4 (66.7%)	4 (66.7%)	1.000
Age (yr)	49.0 (38.5; 54.3)	56.5 (47.8; 58.5)	0.076
BMI (kg/m^2)	25.0 (17.6; 29.3)	28.9 (22.5; 30.4)	0.229
Duration of diabetes (yr)	28.0 (14.8; 35.5)	47.0 (27.0; 50.0)	0.121
HbA1c (mmol/mol)	64.5 (57.8; 72.0)	64.5 (52.5; 72.0)	0.810
Esophageal dysmotility	5 (83.3%)	2 (33.3%)	0.093
Autonomic neuropathy	3 (50.0%)	1 (16.7%)	0.221

Legend: All data are given as median (interquartile range) or number and percentage, using Fisher's exact test and Mann-Whitney U-test. P ≤ 0.05 was considered statistically significant. *Abbreviations*: BMI – body mass index, HbA1c – glycated hemoglobin.

Table 3. Characteristics of patients with and without autonomic neuropathy

Characteristic	Autonomic neuropathy (n = 7)	No autonomic neuropathy (n = 7)	p
Women	2 (28.6%)	7 (100%)	0.007
Age (yr)	54.0 (43.0; 60.0)	55.0 (29.0; 58.0)	0.748
BMI (kg/m^2)	23.5 (17.4; 28.4)	28.4 (23.1; 30.0)	0.116
Duration of diabetes (yr)	33.0 (24.0; 46.0)	17.0 (12.3; 40.0)	0.131
HbA1c (mmol/mol)	63.0 (60.0; 81.0)	61.0 (48.0; 76.0)	0.371
Esophageal dysmotility	3 (42.9%)	4 (57.1%)	0.606
Autonomic neuropathy	3 (42.9%)	2 (28.6%)	0.221

Legend: All data are given as median (interquartile range) or number and percentage, using Fisher's exact test and Mann-Whitney U-test. P ≤ 0.05 was considered statistically significant. *Abbreviations*: BMI – body mass index, HbA1c – glycated hemoglobin.

glucose did not differ significantly between patients with or without gastroparesis. However, patients with gastroparesis had a lower maximal postprandial glucose concentration than those with a normal gastric emptying rate after a fiber-rich breakfast, shown as lower c-max (p = 0.003) and tAUC (p = 0.019), and the change of AUC from baseline (dAUC) tended to be lower (p = 0.093) (**Table 5**).

GIP levels were unaffected by the gastric emptying rate after a fat-rich breakfast (**Table 4**). Both baseline concentration of GLP-1 (p = 0.073) and postprandial concentration of GLP-1 tended to be lower in patients with gastroparesis than in patients with a normal emptying rate with respect to c-max (p = 0.066), d-max (p = 0.073), and tAUC (p = 0.093). Postprandial leptin levels were less decreased (d-min) in patients with gastroparesis

than in patients with normal emptying rate (p = 0.043) (**Table 5**).

A tendency towards lower postprandial concentrations of glucose (tAUC) (p = 0.063) and leptin levels (c-min and tAUC) (p = 0.065 and p = 0.063, respectively) was seen in patients with esophageal dysmotility, when compared with patients with normal esophageal motility (**Table 6**). No significant differences were seen in the concentrations of glucose, GIP, GLP-1, and leptin between patients with and without autonomic neuropathy (**Table 7**).

The basal blood glucose levels prior to the fat-rich breakfast were 11.6 mmol/l (6.6-14.7 mmol/l), and were increased to 12.6 mmol/l (8.3-14.6 mmol/l) at 90 min after the meal. There was no correlation between basal or postprandial glucose levels and peptide levels (data not shown).

3.3 Gut peptide levels in diabetes and health

There were no differences in sex distribution (p = 0.823), age (p = 0.300), and BMI (p = 0.424) between patients with diabetes and the control group. Compared with the healthy control group, patients with diabetes presented with significantly higher basal plasma concentrations of glucose ((9.28 (5.15-11.709) and 5.45 (4.95-5.68) mmol/l, respectively, p = 0.013), GIP (700 (240-1472) and 184 (132-973) pg/ml, respectively, p = 0.023), and leptin (7862 (2086-19492) and 2228 (1220-3208) pg/ml, respectively, p = 0.019)). GLP-1 showed no significant differences in baseline concentration between the groups (225 (28-250) and 163 (131-200) pg/ml, respectively, p = 0.536).

Table 4. Glucose and peptide levels in diabetes patients

Glucose/peptide	Diabetes patients (n = 16)
Glucose	
Baseline (mmol/l)	9.28 (5.15; 11.70)
c-max (mmol/l)	15.40 (11.75; 18.00)
d-max (mmol/l)	4.73 (3.65; 8.31)
tAUC (mmol/l*min)	2400 (1395; 2705)
dAUC (mmol/l*min)	390 (161; 845)
GIP	
Baseline (pg/ml)	700 (240; 1472)
c-max (pg/ml)	3709 (1387; 6183)
d-max (pg/ml)	2906 (544; 5266)
tAUC (ng/ml*min)	458 (176; 808)
dAUC (ng/ml*min)	363 (69; 578)
GLP-1	
Baseline (pg/ml)	225 (28; 250)
c-max (pg/ml)	951 (317; 1122)
d-max (pg/ml)	641 (43; 839)
tAUC (ng/ml*min)	107 (27; 132)
dAUC (ng/ml*min)	61 (10; 90)
Leptin	
Baseline (pg/ml)	7862 (2086; 19492)
c-min (pg/ml)	3704 (1678; 12229)
d-min (pg/ml)	-1998 (-4189; -260)
tAUC (ng/ml*min)	1113 (334; 2495)
dAUC (ng/ml*min)	-75 (-330; 9)

Legend: Glucose levels were measured during a fiber-rich breakfast; plasma peptide levels during a fat-rich breakfast. All data are given as median (interquartile range), using Mann-Whitney U-test. P < 0.05 was considered statistically significant. *Abbreviations*: AUC – area under the curve, c-max – highest measured concentration, c-min – lowest measured concentration, d-max – difference between baseline and c-max, d-min – difference between baseline and c-min, tAUC – total area under the curve, dAUC – AUC from baseline, GIP – glucose-dependent insulinotropic peptide, GLP-1 – glucagon-like peptide 1, n – number.

4. Discussion

This study showed that gastroparesis and autonomic neuropathy are more common in women than in men. This is in accordance with the generally acknowledged higher prevalence of female patients in motility disorders [1]. A single fat-containing meal caused postprandial plasma concentrations of GIP and GLP-1 to increase, whereas leptin concentrations were lowered. Patients with gastroparesis had lower postprandial glucose concentrations after a fiber-rich breakfast, a smaller decrease in postprandial leptin levels, and a tendency to lower basal and postprandial GLP-1 levels after a fat-rich breakfast, when compared with patients with normal gastric emptying rate. The decrease in leptin from baseline and the tAUC of glucose tended to be lower in patients with esophageal dysmotility compared with patients with normal esophageal motility. Autonomic neuropathy was not associated with altered glucose or peptide levels. Furthermore, higher basal plasma concentrations of GIP and leptin were seen in patients with diabetes compared with a healthy control group.

The lower postprandial rise in glucose concentrations in patients with gastroparesis is in accordance with previous studies, and is probably explained by a slower emptying of the nutrients from the ventricle [1]. The tendency towards lower tAUC of glucose in patients with esophageal dysmotility compared with subjects with normal esophageal motility may be explained by a concomitant intestinal dysmotility. Intestinal motility was not measured in the present study, but others

Table 5. Glucose and peptide levels in patients with and without gastroparesis

Glucose / peptide	Gastroparesis (n = 7)	No gastroparesis (n = 9)	p
Glucose			
Baseline	-0.28 (-0.97; 0.50)	0.23 (-0.66; 0.96)	0.366
c-max	-2.35 (-3.53; -1.79)	-0.93 (-1.50; -0.47)	0.003
d-max	-0.10 (0.96; 0.28)	0.04 (-0.43; 0.93)	0.365
tAUC	-0.86 (-1.24; -0.15)	0.69 (-0.08; 1.12)	0.019
dAUC	-0.88 (-0.99; -0.12)	0.13 (-0.09; 1.43)	0.093
GIP			
Baseline	-0.27 (-0.39; 0.58)	-0.07 (-0.98; 0.73)	0.950
c-max	-0.57 (-0.78; 0.49)	0.02 (-0.85; 0.97)	0.681
d-max	-0.47 (-0.65; 0.52)	0.27 (-0.66; 0.96)	0.662
tAUC	-0.57 (-0.67; 1.31)	0.09 (-0.88; 0.66)	1.000
dAUC	-0.42 (-0.54; 0.80)	0.31 (-0.27; 0.78)	0.622
GLP-1			
Baseline	-1.10 (-1.18; 0.22)	0.37 (-0.45; 1.04)	0.073
c -max	-0.71 (-0.91; -0.04)	0.23 (-0.36; 1.27)	0.066
d-max	-0.68 (-0.69; -0.50)	0.17 (-0.07; 1.03)	0.073
tAUC	-1.04 (-1.12-0.18)	0.50 (-0.08-1.07)	0.093
dAUC	-0.87 (-0.90-0.27)	0.43 (-0.39-1.21)	0.127
Leptin			
Baseline	-0.61 (-0.73; -0.02)	0.08 (-0.69; 1.28)	0.414
c-min	-0.35 (-0.52; 1.07)	-0.14 (-0.31; 0.64)	0.536
d-min	0.56 (0.25; 0.57)	0.29 (-1.44; 0.43)	0.043
tAUC	-0.50 (-0.60; 0.32)	-0.21 (-0.66; 0.69)	0.833
dAUC	0.47 (0.23; 0.50)	0.29 (-1.46; 0.47)	0.524

Legend: Glucose levels were measured during a fiber-rich breakfast, plasma peptide levels during a fat-rich breakfast. All data are given as z-scores in median (interquartile range), using Mann-Whitney U-test. $P < 0.05$ was considered statistically significant. *Abbreviations*: AUC – area under the curve, c-max – highest measured concentration, c-min – lowest measured concentration, d-max – difference between baseline and c-max, d-min – difference between baseline and c-min, tAUC – total area under the curve, dAUC – AUC from baseline, GIP – glucose-dependent insulinotropic peptide, GLP-1 – glucagon-like peptide 1, n – number.

have found concordance between dysmotility in the esophagus and small intestine [29].

The digestive gastrointestinal motor function is largely influenced by gut hormones secreted in response to nutrients. Hormones that control postprandial blood glucose concentration play a dominant role in the mechanisms slowing down gastric emptying [1]. GLP-1 and leptin are known to affect gastric emptying [11, 16]. These hormones were influenced by dysmotility in the present study. Since glucose stimulates incretin release [7, 8], the lower GLP-1 concentration in gastroparesis can be explained by the delayed gastric emptying of glucose into the small intestine. On the other hand, the reduced GLP-1 concentration may be a compensatory mechanism to prevent a further decrease of the emptying rate. Such a compensatory downregulating mechanism has previously been described for oxytocin, as oxytocin attenuated gastric emptying in patients with gastroparesis [22, 30]. GLP-1 administration during hyperglycemia has a markedly greater impact on the gastric emptying rate compared with GLP-1 administration during euglycemia [31]. When the hormone levels are analyzed on a single occasion in patients already suffering from gastrointestinal dysmotility, it is difficult to determine these mechanisms, but a compensatory mechanism seems most plausible as baseline levels of GLP-1 were already lowered.

Both patients with gastroparesis and those with esophageal dysmotility showed a smaller decrease in leptin concentrations in response to the meal than those without dysmotility. Leptin secretion has a diurnal rhythm, and the levels have previously been shown to decrease from a peak value early in the morning to lower values later in the day [32]. Also, meal composition affects leptin secretion [33]. Fasting levels of leptin are closely related to the amount of body fat, and leptin impacts on the hypothalamus to reduce food intake, increase energy expenditure, and reduce body weight [13]. As BMI did not differ between the groups, this could not explain the differences in leptin levels between groups.

Leptin slows down gastric emptying to diminish food intake through:

1. Peripheral stimulation of CCK release and thereby afferent vagal activation.
2. A direct central effect on the hypothalamus [16].

There is a positive feedback mechanism mediated by CCK release, and the secreted CCK further enhances leptin secretion [15]. In our previous study, we found that esophageal dysmotility was associated with hyperCCKemia [22]. The elevated CCK levels may explain why the decrease in plasma leptin levels was less pronounced in the present group with esophageal dysmotility.

The diabetes patients in the present study showed a significantly higher basal plasma GIP concentration compared with healthy subjects, while there was no difference in baseline concentration of GLP-1. Similar differences in fasting GIP concentrations, with no difference in GLP-1 levels, have been observed by others, comparing healthy subjects and patients with type 2 diabetes [7] or idiopathic gastroparesis [8]. The observed hormonal plasma changes may be explained by the diminished insulinotropic effect of GIP in diabetes, and either reflects a primary overstimulation of GIP on their receptors, leading to desensitization, or a compensatory mechanism to override the observed GIP receptor dysfunction [6]. Diabetes patients also had higher basal plasma leptin concentrations compared with healthy subjects. This could possibly be explained by the abnormally active renin-angiotensin system seen in diabetes patients, since angiotensin II is a factor that stimulates leptin secretion from adipocytes [34].

Table 6. Glucose- and peptide levels in patients with and without esophageal dysmotility

Glucose / peptide	Esophageal dysmotility (n = 6)	No esophageal dysmotility (n = 6)	p
Glucose			
Baseline	-0.48 (-1.24; 0.62)	0.21 (-0.19; 1.29)	0.240
c-max	-2.20 (-3.61; -1.11)	-0.83 (-2.03; -0.54)	0.240
d-max	-0.53 (-0.97; 0.67)	0.34 (-0.35; 0.73)	0.394
tAUC	-0.86 (-1.24; -0.07)	1.08 (0.11; 1.34)	0.063
dAUC	-0.88 (-0.99; 0.94)	-0.17 (-0.96; 1.40)	0.905
GIP			
Baseline	-0.27 (-0.39; 1.56)	0.16 (-1.08; 0.73)	1.000
c-max	-0.70 (-0.99; -0.05)	0.34 (-58.0; 0.84)	0.310
d-max	0.55 (-1.03; -0.21)	0.45 (-0.55; 0.99)	0.352
tAUC	-0.60 (-1.11; 0.78)	0.62 (-0.68; 0.82)	0.556
dAUC	-0.49 (-1.58; 0.30)	0.45 (-0.13; 0.76)	0.190
GLP-1			
Baseline	-1.10 (-1.18; 0.22)	0.95 (-0.45)*	0.114
c -max	-0.90 (-0.92; 0.50)	-0.01 (-0.66; 0.85)	0.429
d-max	-0.68 (-0.69; -0.50)	0.14 (-1.48)*	0.629
tAUC	-1.05 (-1.12; 0.45)	0.23 (-1.13; 1.34)	0.556
dAUC	-0.87 (-0.90; 0.74)	0.29 (-1.32; 1.15)	0.730
Leptin			
Baseline	-0.61 (-0.73; -0.10)	0.22 (-0.43; 0.78)	0.114
c-min	-0.38 (-0.53; -0.07)	0.64 (-0.23; 1.68)	0.065
d-min	0.56 (-0.24; 0.57)	0.24 (-0.13; 0.43)	0.257
tAUC	-0.57 (-0.60; -0.32)	0.30 (-0.23; 0.82)	0.063
dAUC	0.47 (-0.87; 0.50)	0.30 (0.18; 0.47)	0.730

Legend: Glucose levels were measured during a fiber-rich breakfast, plasma peptide levels during a fat-rich breakfast. All data are given as z-scores in median (interquartile range), using Mann-Whitney U-test. $P < 0.05$ was considered statistically significant. * SPSS did not provide an upper value for the few cases considered. *Abbreviations*: AUC – area under the curve, c-max – highest measured concentration, c-min – lowest measured concentration, d-max – difference between baseline and c-max, d-min – difference between baseline and c-min, tAUC – total area under the curve, dAUC – AUC from baseline, GIP – glucose-dependent insulinotropic peptide, GLP-1 – glucagon-like peptide 1, n – number.

Although the secretion and effects of GIP, GLP-1, and leptin on glucose homeostasis have been widely studied, to our knowledge this is the first study to evaluate these plasma peptide levels in relation to gastrointestinal motility and autonomic neuropathy in type 1 diabetes after a fat-rich meal. Previous studies have measured hormonal effects after glucose load [7] and after fat load [19, 20] in healthy subjects and type 2 diabetes. The role of these peptides in type 1 diabetes after the meal is less well defined. The focus of research into gastrointestinal dysmotility in diabetes has been on gastroparesis to date; esophageal dysmotility is rarely considered.

A limitation of the present study is the small number of subjects, but the patients were thoroughly examined, which is an asset. The study shows that gastrointestinal motility should be considered in all efforts to analyze peptide levels, and that the degree of dysmotility affects the peptide levels within an identified disease. While a fat load in diabetic gastroparesis affected plasma levels of GLP-1 and not GIP in our study, the hormonal response to a glucose load in idiopathic gastroparesis has been shown to affect the plasma levels of GIP and not GLP-1 [8]. This shows that a nutritional composition as part of a diet or medical nutritional therapy should be examined carefully in health and disease.

There is some debate about the question of which food is most suitable in diabetes. Three different Nordic diabetes diets and the Mediterranean diet are currently recommended [35-37]. The

Table 7. Glucose and peptide levels in patients with and without autonomic neuropathy

Glucose/ peptide	Autonomic neuropathy (n = 7)	No autonomic neuropathy (n = 7)	p
Glucose			
Baseline	0.50 (-1.25; 0.99)	0.07 (-0.69; 0.77)	0.875
c-max	-1.56 (-2.47; -1.00)	-1.79 (-2.75; -0.93)	1.000
d-max	-0.45 (0.96; -0.10)	0.08 (-0.10; 0.40)	0.078
tAUC	-0.10 (-1.15; 0.61)	0.37 (-0.99; 1.23)	0.699
dAUC	-0.09 (-1.00; 0.26)	-0.17 (-0.91; 0.63)	0.818
GIP			
Baseline	-0.42 (-1.03; -0.20)	0.09 (-0.36; 0.44)	0.138
c-max	-0.42 (-0.90; 1.50)	0.25 (-0.61; 0.72)	0.620
d-max	-0.05 (-0.72; 0.55)	0.31 (-0.50; 0.67)	0.628
tAUC	-0.00 (-0.69; 0.84)	0.16 (-0.64; 0.77)	0.818
dAUC	0.40 (-0.34; 0.94)	0.05 (-0.46; 0.64)	0.485
GLP-1			
Baseline	0.16 (-0.83; 0.44)	0.95(-1.10; 1.22)	0.429
c -max	-0.48 (-1.11; 0.31)	0.23 (-0.90; 0.90)	0.366
d-max	-0.26 (-0.89; 0.58)	0.14 (0.68; 0.95)	0.662
tAUC	-0.02 (-1.23; 0.49)	0.23 (-1.06; 1.31)	0.394
dAUC	-0.25 (-1.14; 0.38)	0.29 (-0.87; 0.88)	0.310
Leptin			
Baseline	-0.58 (-0.76; -0.28)	0.22 (-0.66; 0.95)	0.295
c-min	-0.10 (-0.35; 0.56)	-0.14 (-0.41; 1.07)	0.902
d-min	0.45 (0.32; 0.14)	0.23 (-0.79; 0.57)	0.628
tAUC	-0.43 (-0.76; 0.54)	0.04 (-0.52; 0.80)	0.310
dAUC	0.42 (0.26; 0.53)	0.30 (-0.43; 0.49)	0.394

Legend: Glucose levels were measured during a fiber-rich breakfast, plasma peptide levels during a fat-rich breakfast. All data are given as z-scores in median (interquartile range), using Mann-Whitney U-test. $P < 0.05$ was considered statistically significant. *Abbreviations*: AUC – area under the curve, c-max – highest measured concentration, c-min – lowest measured concentration, d-max – difference between baseline and c-max, d-min – difference between baseline and c-min, tAUC – total area under the curve, dAUC – AUC from baseline, GIP – glucose-dependent insulinotropic peptide, GLP-1 – glucagon-like peptide 1, n – number.

present study shows that the response to food does not only depend on nutritional composition, but also on gastrointestinal motility. Patients with dysmotility are recommended to eat mashed food to improve gastric emptying rate. The present study also suggests that the composition of food should depend on the presence or absence of gastrointestinal dysmotility to improve the hormonal profile. Altered plasma levels of GIP and leptin, as observed in diabetes, may have deleterious effects on the disease [6, 18]. Consequently, the role of gastrointestinal dysmotility in diabetes is important for the development of optimal diet recommendations, and deserves further examination.

5. Conclusions

A pure fat-containing meal caused a postprandial increase in plasma concentrations of GIP and GLP-1, whereas leptin concentrations were lowered. No differences were seen in baseline concentrations of glucose in patients with or without gastroparesis, but gastroparesis was associated with lower postprandial glucose concentrations after a fiber-rich breakfast, a smaller decrease in postprandial leptin levels, and a tendency to lower basal and postprandial plasma levels of GLP-1 after a fat-rich breakfast, compared with patients with normal gastric emptying. Esophageal dysmotility was associated with a tendency towards a lower postprandial glucose concentration, a reduced lowering of plasma leptin levels from baseline, and a lower tAUC. Higher basal plasma levels of GIP and leptin were seen in patients with diabetes compared with a healthy control group.

Acknowledgments: This study was sponsored by Bengt Ihre Foundation, Development Foundation of Region Skane, and King Gustav 5th and Queen Victoria's Free Mason's Foundation.

Disclosures: The authors report no conflict of interests.

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