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Effects of different metabolic states and surgical models on glucose metabolism and secretion of ileal L-cell peptides: a study protocol for a cross-sectional study

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A Celik has nothing to disclose

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ABSTRACT

Introduction: Obesity and type 2 diabetes mellitus (T2DM) are increasing worldwide, thus reaching pandemic proportions. The role functional restriction and gut hormones can be a beneficial tool in treating obesity and diabetes. However, the exact hormonal profiles in different metabolic states and surgical models are unknown.

Methods and analysis: The HIPER-1 study is a single centre cross sectional study in which a total 240 (in different metabolic states and surgical models) will receive an Oral Mixed Meal Tolerance Test (OMTT). At baseline and after 30, 60 and 120 minutes the PYY levels, GLP-1 levels, glucose and insulin sensitivity will be measured. The primary endpoint of the study will be the area under the GLP-1 and Peptide – YY curves following the OMTT. Secondary study endpoints include: examination of the difference in the plasma levels of the distal ileal hormones in subjects with various health statuses and in patients who have been treated by different surgical techniques.

Ethics and dissemination An independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board approved the study protocol. Dissemination will occur via publication, national and international conference presentations, and exchanges with regional, provincial and national stakeholders.

Trial registration number: NCT02532829

Strengths and limitations of this study

Strengths:

- The HIPER-1 study gives new insight in gut hormone profiles in different metabolic states and surgical models
- The HIPER-1 study gives insight in glucose metabolism and insulin resistance and its correlation with the gut hormones

Limitations:

- This study is limited to four surgical procedures: sleeve gastrectomy, mini gastric bypass, sleeve gastrectomy with ileal transposition and sleeve gastrectomy with transit bipartition.
- This is a single centre study performed in a Turkish metabolic surgery clinic with a specific patient population, which may give problems in terms of generalisability of the study results.

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are increasing worldwide, thus reaching pandemic proportions [1]. Diet, exercise and medication remain the cornerstones of type 2 diabetes mellitus treatment. But, apart from studies demonstrating promising results in some of the developed countries; the long-term success rates of lifestyle and drug modifications are disappointing [2]. Despite an impressive armamentarium of pharmacotherapeutics, adequate long-term glycemic control is difficult, and overly tight glycemic control introduces a proportionate risk of hypoglycemia and targets have been modified because of the high risk of cardiovascular events [3]. Moreover, diabetes medication can promote weight gain, which in turn exacerbates the obesity issues [4 5].

In cases where classic strategies proved to be inadequate, broad type of gastrointestinal (GI) surgery methods offer new alternatives to treat obesity and T2DM [6]. Among severely obese patients, bariatric surgical options cause significant sustained weight loss, improve obesity-related co-morbidities, and reduction in long-term mortality [7]. Currently, bariatric surgery is considered to be appropriate for individuals with a body mass index (BMI) >35 kg/m^2 and serious obesity-related comorbidities, including T2DM. Operations involving intestinal bypasses exert particularly higher effects on diabetes [8]. Mounting evidence indicates that these remarkable effects result not only from weight loss but also from weight-independent anti-diabetic mechanisms [9]. Consequently, conventional bariatric procedures and new experimental GI operations are being explored for the management of patients with T2DM and are overweight or class I obese (BMI: 30-35 kg/m^2).

Many physiological mechanisms proposed to explain the improvement of glucose metabolism, insulin metabolism and beta-cell function following surgery include:

a) Major diet restriction early after surgery,
b) Hepatic insulin sensitivity recovery early after surgery,
c) Increase of incretin hormone (GLP-1) caused by rearrangements in the gastro-intestinal tract,
d) The earlier blockage of glucagon secretion, caused by GLP-1,
e) Less hunger and early satiety (changes in Ghrelin, GLP-1, PYY and Oxyntomodulin (OXM)
f) Recovery of Beta-cell function by incretins stimulation and
g) Weight loss induced reduction in beta-cell gluco- and lipotoxicity [10-14].

Evidence gaps to be filled

The complete mechanisms of glucose metabolism, insulin metabolism and the changes after metabolic surgery remain poorly understood. The variable levels of incretin stimulation (especially GLP-1) and improved glycaemic control in those with diabetes have been shown following various bariatric techniques [10-13]. PYY1-36 is also synthesized and released from specialized entero-endocrine cells called L-cells found predominantly within the distal GI tract (hindgut) and is then cleaved by the enzyme DPP-4 to give the active form, PYY3-36 [15]. In our study, we will measure the active PYY3-36 to better document the effects of active form of PYY for 8 groups. The measurement of serum PYY in No Surgery and Surgery Groups will give us the pattern of PYY stimulation in different groups. Since all 8 groups will be tested with the standard Oral Mixed Meal Tolerance Test (OMTT) the macronutrients effect on PYY peak will be overcome [16]. “Ileal brake” term can be considered as a summary of GLP-1 and PYY actions on gut including reduction in gastric emptying and a delay in intestinal transit, which can be used as a good tool for the treatment of people with obesity and related conditions. We must add that they also act on both peripheral and central nervous systems concentrating at the arcuate nucleus of the hypothalamus (ARC), which plays a key role in the regulation of appetite. Batterham et al. [17] published its effects on rats and next year

documented the PYY effects on humans [18] indicating that the basal levels of PYY in obese subjects when compared with normal weight subjects were lower. There was also a blunted postprandial PYY rise suggesting that a lack of endogenous PYY secretion may be implicated in the development of obesity. In our study, we expect to see different patterns of serum PYY levels to better explain its role in the 8 different groups. GLP-1 is secreted from distal intestinal L-cells along with Peptide YY and Oxyntomodulin in response to a meal. But little is known about the levels of distal intestinal L-cell hormones in healthy individuals, different disease states, and different body compositions. Also, the difference about the baseline values and activities of these hormones after different surgical techniques has not been extensively studied. The present study will give insight in the physiology of these gut hormones and its relation to the glucose metabolism after metabolic surgery.

SPECIFIC AIMS:
We plan to test our hypothesis and, thereby accomplish the objective of this application by pursuing the following specific aims:

Aim 1: To measure and compare the levels of GLP-1 and Peptide YY in non-obese healthy volunteers vs. obese diabetics vs. obese non-diabetics vs. non-obese diabetics with administration of a standardized OMTT at baseline, 30-60-120 minutes.
Hypothesis 1: There will be increasing levels of GLP-1 and Peptide YY responses to a OMTT in individuals across diabetes-obesity spectrum; from those who are obese diabetics followed by non-obese diabetics, obese non-diabetic and healthy non-obese non-diabetics.

Aim 2: To measure and compare the levels of GLP-1 and Peptide YY in patients who have undergone sleeve gastrectomy (SG) vs. Mini-gastric bypass (MGB) vs. Sleeve gastrectomy with Ileal Transposition (SIT) vs. Sleeve gastrectomy with Transit Bipartition (STB) after administration of OMTT at baseline, 30-60-120 minutes.
Hypothesis 2: There will be increasing levels of GLP-1 and Peptide YY following an OMMT in patients who have undergone a SG followed by MGB, SIT and STB.

Aim 3: To analyze the response of insulin and glucose following the OMTT in relation to the type of surgery.
Hypothesis 3: There will be marked improvements in glycaemic control and insulin activity in techniques with bowel anastomosis, compared to SG.

In this study, we aimed to analyse the baseline levels and 30-60-120 min postprandial activities of GLP-1, and Peptide YY in No Surgery and Surgery Groups (defined below). This will be the initial evaluation of a durability study that is planned for a minimum of 5 years follow up.

METHODS
This cross-sectional study will be performed at the Metabolic Surgery Clinic in Istanbul. Inclusion will be performed by the physician researcher after written informed consent. The study consists of a non-surgical and a surgical group.

Sample size calculation and statistical analysis
The study is fashioned as a cross-sectional analysis and it will be an IRB approved prospective study. Sample size calculation is based on the formula of Kelsey. The sample size will be 120 subjects in the surgical group and 120 subjects in the non-surgical group (which

means 30 subjects in each subgroup) based on a significance level of 5%, a power of 80% and a mean decrease of fasting glucose level (postoperative) of 35%. [19 20]
In total of 240 patients will be included in this study (table 2). Continuous variables will be presented as mean ± standard deviation (SD). Categorical variables were presented as frequency with percentages. Statistical analysis will be performed by Repeated Measurement Analysis of Variance and 1-way ANOVA.

In all tests, values of $p<0.05$ were considered statistically significant. Statistical Package for Social Sciences (SPSS, Chicago, IL, USA Version 20.0) will be used to prepare the database and for statistical analysis.

1. Non-Surgical Group:

1.1 Study population
We will be studying 120 subjects (aged 30 to 60 years) in the non-surgical groups.

1.2 Inclusion criteria:

a. For healthy subjects (GROUP NS-A): No known disease, no previous surgery, HbA1c<5.7%, BMI<25 kg/m^2 (n=30)
b. For diabetic obese (GROUP NS-B): Type 2 diabetes diagnosis longer than 3 years; BMI>30 kg/m^2 (n=30)
c. For diabetic non-obese (GROUP NS-C): Type 2 diabetes diagnosis longer than 3 years; BMI<30 kg/m^2 (n=30)
d. For obese non-diabetics (GROUP NS-D): HbA1c<5.7%, No signs and history of T2D, and BMI>30 kg/m^2 (n=30)

1.3 Exclusion criteria:

a. Anti insulin / islet antibody and glutamic acid decarboxylase antibody (antiGAD) positivity, plasma fasting C-peptide lesser than 1 ng/ml.
b. Liver cirrhosis, severe renal failure, collagen diseases, severe endocrinopathies, blindness.
c. Heart failure, acute myocardial infarction, stroke or transient ischemic attack, unstable angina pectoris.
d. History of malignancy or malignant neoplasm in place, severe inflammatory complications, neurological or cardiovascular in act.
e. Pregnancy
f. Any conditions that at the discretion of the head of the study can represent risk to the patient or could affect the protocol results.

1.4 Recruitment of the subjects
The subjects for this non-surgical arm of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility. Patients who accept to stop their anti-diabetic medications 2 days prior to evaluation will be enrolled in the study.

2. Surgical Group:

2.1 Identification of study population

In the Surgical Group there are 4 different types of surgery. Age and sex matched patients who underwent laparoscopic sleeve gastrectomy (Group SG), mini-gastric bypass (Group MGB), sleeve gastrectomy with ileal transposition (Group IT) and sleeve gastrectomy with transit bipartition (Group TB) operated not less than 6 months, but within the last 2 years will be enrolled. The primary methodology of the study is to achieve adequate number of patients via an announcement through the website of Turkish Metabolic Surgery Foundation and divide the patients due to the mentioned categories.

In the surgery groups, we expect a change of the hormones by proximalising an intestinal limb and as a consequence activating the entero-insular axis. Similarly as observed in morbidly obese subjects after bariatric surgery, changes will be stable in the long time. Based in observations in morbidly obese and T2DM morbidly obese subjects we expect great reduction or disappearance of insulin resistance (IR) and improvement of beta-cell function, represented here by parameters obtained from mathematical model applied on MMT data (Fasting insulin secretion, Total insulin secretion, beta-cell glucose sensitivity, rate sensitivity and potentiation factor) with consecutive improvement of clinical T2DM symptoms and of the others components of the metabolic syndrome.

To assess the characteristics of distal ileal hormones we will perform an oral mixed meal tolerance test (OMTT), and analyze the parameters in Box 1. The exclusion criteria are the same as in the Non-surgical group.

2.2 Inclusion criteria

a. Type 2 Diabetic patients who underwent a sleeve gastrectomy, a mini-gastric bypass, a sleeve gastrectomy with ileal transposition or a sleeve gastrectomy with transit bipartition performed more than 6 months ago, but within the last 2 years, with steady weight profile.
b. Preferably not on any kind of anti-diabetic drugs or will accept cessation of all anti-diabetic drugs 2 days prior to evaluation.
c. Absence of or resolved co-morbidities (dyslipidemia, hypertension, neuropathy, retinopathy, cardiovascular disease, stroke events or lower extremity amputation).
d. Possibility to participate to the quadruplicate measurement protocol.

2.3 Recruitment of the subjects

The subjects for this non-surgical arm of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility. Patients who accept to stop their anti-diabetic medications 2 days prior to evaluation will be enrolled in the study.

3. Intervention

Oral Mixed Meal Tolerance Test (OMTT): A standard mixed meal tolerance test (350 kcal, consisting of 55% carbohydrate, 25% protein, and 20% fat) is going to be performed in each participant. Venous blood samples will be collected at fasting stage and at 30, 60 and 120 minutes after the OMTT via catheter localized in ante-cubital vein. All blood samples will be drawn according to OMTT protocol (See Table 1).

4. Analytical Procedures

All the blood samples will be collected according to aforementioned OMTT protocol. Blood samples collected into ice-chilled tubes containing K2EDTA (spray-dried) tubes treated with DPP-4 inhibitor (BD Cat No: 366473 vacutainer® P700) will be used for PYY and, GLP-1 determinations. The tubes will be kept on ice until centrifuged in +4oC for 20 min at 4000 g. Plasma will be separated and kept frozen at -20oC immediately in aliquots of 300 ml until analysis. Serum separated by gel containing yellow levander tubes for the analysis of SGOT, SGPT, GGT, and whole blood samples collected into EDTA Na2 for HbA1c analysis are going to be used. Plasma glucose will be monitored from the plasma obtained from Fluoro-oxalate tubes (grey levander). Plasma insulin will be measured from plasma obtained from EDTA Na2 tubes. Liver function tests (SGOT, SGPT, and GGT) and HbA1c will have a single measurement during fasting. Plasma insulin levels and plasma glucose levels will be measured during fasting and 30-60-120 minutes after OMTT.

5. Outcomes Measured

During the visit a complete medical history and physical exam will be performed. Body weight, waist and hip circumference and Body Mass Index will be measured and recorded. The following outcomes (Box 1) will also be measured:

a. Plasma PYY will be measured by commercial ELISA kit of Biovender Research and Diagnostics products "Human PYY ELISA" Cat No: RSCYK080R with a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. The EIA kit shows 100% cross reactivity to human PYY (3-36) and human PYY (1-36), and shows less than 0.003% cross reactivity to human and rat NPY, which have similar amino acid sequence with human PYY.

Test Principle: This EIA kit for determination of human PYY in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. To the wells of plate coated with rabbit anti human PYY antibody, standard or samples, labeled antigen are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled streptoavidin-biotinylated antigen-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3'.

b. Plasma total GLP-1 will be measured by commercial ELISA kit of DRG® "GLP-1 (total) (EIA-5095) with a two-site "sandwich" technique with two selected GLP-1 antibodies. *Test Principle*: This ELISA is designed, developed and produced for the quantitative measurement of GLP-1 (7-36) and (9-36) in plasma sample. The assay utilizes the two-site "sandwich" technique with two selected GLP-1 antibodies. Assay standards, controls and test samples are directly added to wells of a microplate that is coated with streptavidin. Subsequently, a mixture of biotinylated GLP-1 specific antibody and a horseradish peroxidate (HRP) conjugated GLP-1 specific antibody is added to each well. After the first incubation period, a "sandwich" immunocomplex of "Streptavidin – Biotin-Antibody – GLP-1(7-36)/(9-36) – HRP conjugated antibody" is formed and attached to the wall of the plate. The unbound HRP conjugated antibody is removed in a subsequent washing step. For the detection of this immunocomplex, each well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the immunocomplex bound to GLP-1 (7-36)/(9-36) on the wall of the microtiter well is directly proportional to the amount of Total GLP-1 in the sample.

i. Sensitivity The sensitivity of this Total GLP-1 ELISA as determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.6 pmol/L.
ii. Specificity This Bioactive GLP-1 (7-36) assay is specific measure GLP-1 (7-36). It is expected that this assay does not detect following peptides.

1. GLP-1 (7-36) 100%
2. GLP-1 (9-36) 100%
3. GLP-1 (9-37) < 0.1%
4. GLP-1 (7-37) < 0.1%
5. GLP-1 (1-36) < 0.1%
6. GLP-2 < 0.1%
7. Glucagon < 0.1%

c. Liver Function Tests: Liver function tests (SGOT, SGPT, and GGT) will be measured by IFCC Enzymatic Assay in a Cobas 6000, Roche Diagnostics.

d. HbA1c: HbA1c will be measured by the turbidometric assay of Tina-quant Hemoglobin A1c Gen3 in a Cobas 6000 based on measurement of antipolyhapten complex.

e. Plasma Insulin Levels: will be measured by the ECLIA of Insulin Cobas 6000 based on sandwich assay. For quality control, PreciControl Multimarker or PreciControl Universal are going to be used.

f. Plasma Glucose Levels: will be measured by Enzymatic reference method with hexokinase. Hexokinase catalyzes the phosphorylation of glucose to glucose 6 phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrical.

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H+}$$

6. Primary Endpoints

The primary endpoint as the key outcome measure of the study will be area under the GLP-1, Peptide – YY, glucose and insulin curves following the OMTT.

7. Secondary Endpoints

Secondary study endpoints include: examination of the difference in the plasma levels of the distal ileal hormones in subjects with various health statuses and in patients who have been treated by different surgical techniques.

8. Ethics and informed consent

An independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board approved the study protocol. Oral and written informed consent from the patient will be obtained prior to inclusion.

9. Adverse Events

Although the Oral Mixed Meal Tolerance Test (OMTT) is considered safe, serious adverse events possibly related to the OMTT will be reported to the ethical committee

Ethics and dissemination

We have obtained ethical approval from an independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board. Oral and written informed consent from the patient will be obtained prior to inclusion. This study will take place in the Metabolic Surgery Clinic, Sisli, Istanbul, Turkey and the inclusion of patients will take place between Oktober 2015 and March 2016.
The subjects for the non-surgical group of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility.
The subjects for the surgical group there are 4 different types of surgery. Age and sex matched patients who underwent laparoscopic sleeve gastrectomy (Group SG), mini-gastric bypass (Group MGB), sleeve gastrectomy with ileal transposition (Group IT) and sleeve gastrectomy with transit bipartition (Group TB) operated not less than 6 months, but within the last 2 years will be enrolled. Via an announcement through the website of Turkish Metabolic Surgery Foundation patients will be recruited and divided in the above mentioned.
The OMTT will be done by educated lab personnel, as well as the measurements of PYY, GLP-1 and oxyntomodullin.

The research nurses will collect the necessary data (from patient charts) and will store them on a secure harddrive. These harddrives will be collected and stored at the Metabolic Surgery Clinic in Istanbul in Turkey.

Dissemination plan

We expect that the results of this study, which will help us to understand the physiology regarding the enteric gut hormones and the improvement of glucose metabolism and insulin sensitivity after bariatric and metabolic surgery. The results will also give us insight in how to select patients for the specific bariatric surgical procedures. We expect the study to have some international appeal because of the increasing interest in gut hormone physiology and its correlation with patient outcomes (in terms of improvement/remission of type 2 diabetes after surgery). For end-of-study knowledge dissemination we intend to publish in medical, health services and/or public health journals. More importantly, we plan to present and discuss the results of our study on national and international congresses, focussing on surgery and endocrinology.

DISCUSSION

In this study we have three main hypotheses; 1) There will be increasing levels of GLP-1 and Peptide YY responses to a OMTT in individuals across diabetes-obesity spectrum; from those who are obese diabetics followed by non-obese diabetics, obese non-diabetic and healthy non-obese non-diabetics, 2) There will be increasing levels of GLP-1 and Peptide YY following a MMT in patients who have undergone a SG followed by MGB, SIT and STB, and 3) There will be marked improvements in glycemic control and insulin activity in techniques with bowel anastomosis, compared to SG. To our knowledge, this is the first study that aims to extensively research the glucose metabolism and secretion of ileal L-cell peptides in different metabolic states and surgical models.

There is increasing evidence that gut hormones play an important role in the neuro-endocrine physiology of hunger and satiety. As pointed out by Santoro et al. [21] and Celik et al. [19 20] there is need for a change in the current practice of bariatric/metabolic surgery. For these changes we have to focus on functional restriction, the proximal and distal gut imbalance and the role of gut hormones like, PYY, GLP-1 and oxyntomodulin [19 21].

One of the important hormones in the proximal gut is GIP (glucose-dependent insulinotropic polypeptide) that produces an insulinic response, but instead of decreasing the secretion of glucagon, it enhances it [21]. In obese and diabetic patients there are abnormally high levels of GIP present (mainly a proximal gut product) [22]. Any kind of dietary restriction will lead to significant decreases in the GIP levels [23]. GIP is a hormone that is obesogenic and insulinotropic and strategies to block GIP production are beneficial for these patients [24 25].

The opposite, the distal gut hormones (GLP-1, PYY and oxyntomodulin) or their agonists are beneficial for obese and diabetics as well. Either way, blocking the hormonal activity of the proximal gut and increasing the activity of the distal gut, is beneficial. Surgical procedures support these findings [20]. Because of the sparse literature on the production of earlier mentioned hormones in different metabolic states and surgical models, a total of eight groups will be compared with each other (see table 2), to gain more insight in the physiology of glucose and hormonal metabolism.

TABLE 1: Oral Mixed Meal Tolerance Test (OMTT) Protocol

	Yellow levander with gel separator	Na2 EDTA	2 X K2 EDTA +DPP IV inhibitor	
Time				**Volume**
0	**7**	**3**	**6**	**16**
30	**7**	**3**	**6**	**16**
60	**7**	**3**	**6**	**16**
120	7	3	6	**16**

- **Yellow Levander with Gel Separator:** SGOT, SGPT, GGT
- **Na2EDTA:** = HbA1c
- **2xK2EDTA + DPP IV inhibitor:** Glucagon Like Peptide-1 (GLP-1); Peptide YY

TABLE 2: Overview of study groups and outcomes measured

NON-SURGICAL GROUPS (n=120)

1. Healthy volunteers (n=30)
2. Obese diabetics (n=30)
3. Obese non-diabetics (n=30)
4. Non-obese diabetics (n=30)

SURGERY GROUPS (n=120)

5. Sleeve Gastrectomy (SG, n=30)
6. Mini Gastric Bypass (MGB, n=30)
7. Sleeve Gastrectomy with Ileal Transposition (IT, n=30)
8. Sleeve Gastrectomy with Transit Bipartition (TB, n=30)

Box 1: Outcomes Measured:

* Baseline and 30-60-120 minutes GLP-1, and Peptide YY response to an OMTT,
* Baseline and 30-60-120 minutes plasma insulin and glucose measurements,
* Fasting lipid profile: total cholesterol, HDL and LDL-cholesterol and triglycerides,
* Liver profile: AST, ALT and GGT,
* Body weight, BMI, waist and hip circumference

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Effects of different metabolic states and surgical models on glucose metabolism and secretion of ileal L-cell peptides: a study protocol for a cross-sectional study

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Effects of different metabolic states and surgical models on glucose metabolism and secretion of ileal L-cell peptides: a study protocol for a cross-sectional study

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ABSTRACT

Introduction: Obesity and type 2 diabetes mellitus (T2DM) are increasing worldwide, thus reaching pandemic proportions. The role functional restriction and gut hormones can be a beneficial tool in treating obesity and diabetes. However, the exact hormonal profiles in different metabolic states and surgical models are unknown.

Methods and analysis: The HIPER-1 study is a single centre cross sectional study in which a total 240 (in different metabolic states and surgical models) will receive an Oral Mixed Meal Tolerance Test (OMTT). At baseline and after 30, 60 and 120 minutes the PYY levels, GLP-1 levels, glucose and insulin sensitivity will be measured. The primary endpoint of the study will be the area under the GLP-1 and Peptide – YY curves following the OMTT. Secondary study endpoints include: examination of the difference in the plasma levels of the distal ileal hormones in subjects with various health statuses and in patients who have been treated by different surgical techniques.

Ethics and dissemination An independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board approved the study protocol. Dissemination will occur via publication, national and international conference presentations, and exchanges with regional, provincial and national stakeholders.

Trial registration number: NCT02532829

Strengths and limitations of this study

Strengths:

- The HIPER-1 study gives new insight in gut hormone profiles in different metabolic states and surgical models
- The HIPER-1 study gives insight in glucose metabolism and insulin resistance and its correlation with the gut hormones

Limitations:

- This study is limited to four surgical procedures: sleeve gastrectomy, mini gastric bypass, sleeve gastrectomy with ileal transposition and sleeve gastrectomy with transit bipartition.
- This is a single centre study performed in a Turkish metabolic surgery clinic with a specific patient population, which may give problems in terms of generalisability of the study results.
- Utilisation of only a 2-day washout period for diabetes medications.

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are increasing worldwide, reaching pandemic proportions [1]. Diet, exercise and medication remain the cornerstones for the treatment of T2DM. But, apart from studies demonstrating promising results in some of the developed countries; the long-term success rates of lifestyle and drug modifications are disappointing [2]. Even with an impressive armamentarium of medication, adequate long-term glycemic control is difficult, and overly tight glycemic control introduces a proportionate risk of hypoglycemia and targets have been modified because of the high risk of cardiovascular events [3]. Moreover, diabetes medication can promote weight gain, which in turn exacerbates the obesity issues [4 5].
In cases where classic strategies proved to be inadequate, broad type of gastrointestinal (GI) surgical methods offer new alternatives to treat obesity and T2DM [6]. Among severely obese patients, bariatric surgical options cause significant sustained weight loss, improvement of obesity-related co-morbidities, and reduction in long-term mortality [7]. Currently, bariatric surgery is considered to be appropriate for individuals with a body mass index (BMI) >35 kg/m^2 and serious obesity-related comorbidities, including T2DM. Surgical procedures involving intestinal bypasses exert particularly higher effects on diabetes [8]. Mounting evidence indicates that these remarkable effects result not only from weight loss but also from weight-independent anti-diabetic mechanisms [9]. Consequently, conventional bariatric procedures and new experimental GI operations are being explored for the management of patients with T2DM and are overweight or class I obese (BMI: 30-35 kg/m^2).
Many physiological mechanisms proposed to explain the improvement of glucose metabolism, insulin metabolism and beta-cell function following surgery include:
a) Major diet restriction early after surgery,
b) Hepatic insulin sensitivity recovery early after surgery,
c) Increase of incretin hormone (GLP-1) caused by rearrangements in the gastro-intestinal tract,
d) The earlier blockage of glucagon secretion, caused by GLP-1,
e) Less hunger and early satiety (changes in Ghrelin, GLP-1, PYY and Oxyntomodulin (OXM)
f) Recovery of Beta-cell function by incretins stimulation and
g) Weight loss induced reduction in beta-cell gluco- and lipotoxicity [10-14].

Evidence gaps to be filled

The complete mechanisms of glucose metabolism, insulin metabolism and the changes after metabolic surgery remain poorly understood. The variable levels of incretin stimulation (especially GLP-1) and improved glycaemic control in those with diabetes have been shown following various bariatric techniques [10-13]. PYY1-36 is also synthesized and released from specialized entero-endocrine cells called L-cells found predominantly within the distal GI tract (hindgut) and is then cleaved by the enzyme DPP-4 to give the active form, PYY3-36 [15]. In our study, we will measure the active PYY3-36 to better document the effects of active form of PYY for 8 groups. The measurement of serum PYY in No Surgery and Surgery Groups will give us the pattern of PYY stimulation in different groups. Since all 8 groups will be tested with the standard Oral Mixed Meal Tolerance Test (OMTT) the macronutrients effect on PYY peak will be overcome [16]. “Ileal brake” term can be considered as a summary of GLP-1 and PYY actions on gut including reduction in gastric emptying and delay in intestinal transit, which can be used as a good tool for the treatment of people with obesity and related conditions. We must add that they also act on both peripheral and central nervous systems concentrating at the arcuate nucleus of the hypothalamus (ARC), which plays a key role in the regulation of appetite. Batterham et al. [17] published its effects on rats and next year documented the PYY effects on humans [18] indicating that the basal levels of PYY in obese

subjects when compared with normal weight subjects were lower. There was also a blunted postprandial PYY rise suggesting that a lack of endogenous PYY secretion may be implicated in the development of obesity. In our study, we expect to see different patterns of serum PYY levels to better explain its role in the 8 different groups. GLP-1 is secreted from distal intestinal L-cells along with Peptide YY and Oxyntomodulin in response to a meal. But little is known about the levels of distal intestinal L-cell hormones in healthy individuals, different disease states, and different body compositions. Also, the difference about the baseline values and activities of these hormones after different surgical techniques has not been extensively studied. The present study will give insight in the physiology of these gut hormones and its relation to the glucose metabolism after metabolic surgery. Secondly this study will give insight in differences between gut hormone levels in different metabolic states and after different surgical procedures, which will be necessary in understanding physiological aspects of these gut hormones.

SPECIFIC AIMS:
We plan to test our hypothesis and, thereby accomplish the objective of this application by pursuing the following specific aims:

Aim 1: To measure and compare the levels of GLP-1 and Peptide YY in non-obese healthy volunteers vs. obese diabetics vs. obese non-diabetics vs. non-obese diabetics with administration of a standardized OMTT at baseline, 30-60-120 minutes.
Hypothesis 1: There will be increasing levels of GLP-1 and Peptide YY responses to a OMTT in individuals across diabetes-obesity spectrum; from those who are obese diabetics followed by non-obese diabetics, obese non-diabetic and healthy non-obese non-diabetics.

Aim 2: To measure and compare the levels of GLP-1 and Peptide YY in patients who have undergone sleeve gastrectomy (SG) vs. Mini-gastric bypass (MGB) vs. Sleeve gastrectomy with Ileal Transposition (SIT) vs. Sleeve gastrectomy with Transit Bipartition (STB) after administration of OMTT at baseline, 30-60-120 minutes.
Hypothesis 2: There will be increasing levels of GLP-1 and Peptide YY following an OMMT in patients who have undergone a SG followed by MGB, SIT and STB.

Aim 3: To analyze the response of insulin and glucose following the OMTT in relation to the type of surgery.
Hypothesis 3: There will be marked improvements in glycaemic control and insulin activity in techniques with bowel anastomosis, compared to SG.

In this study, we aimed to analyse the baseline levels and 30-60-120 min postprandial activities of GLP-1, and Peptide YY in No Surgery and Surgery Groups (defined below). This will be the initial evaluation of a durability study that is planned for a minimum of 5 years follow up.

METHODS
This cross-sectional study will be performed at the Metabolic Surgery Clinic in Istanbul. Inclusion will be performed by the physician researcher after written informed consent. The study consists of a non-surgical and a surgical group.

Sample size calculation and statistical analysis
The study is fashioned as a cross-sectional analysis and it will be an IRB approved prospective study. Sample size calculation is based on the formula of Kelsey. The sample size

will be 120 subjects in the surgical group and 120 subjects in the non-surgical group (which means 30 subjects in each subgroup) based on a significance level of 5%, a power of 80% and a mean decrease of fasting glucose level (postoperative) of 35%. [19 20]
In total of 240 patients will be included in this study (table 1). Continuous variables will be presented as mean ± standard deviation (SD). Categorical variables were presented as frequency with percentages. Statistical analysis will be performed by Repeated Measurement Analysis of Variance and 1-way ANOVA.

In all tests, values of $p<0.05$ were considered statistically significant. Statistical Package for Social Sciences (SPSS, Chicago, IL, USA Version 20.0) will be used to prepare the database and for statistical analysis.

1. Non-Surgical Group:

1.1 Study population
We will be studying 120 subjects (aged 30 to 60 years) in the non-surgical groups.

1.2 Inclusion criteria:

a. For healthy subjects (GROUP NS-A): No known disease, no previous surgery, HbA1c<5.7%, BMI<25 kg/m^2 (n=30)
b. For diabetic obese (GROUP NS-B): Type 2 diabetes diagnosis at least longer than 3 years; under stable medical treatment; HbA1c >7%; weight stability, defined as no significant change (5%) within the last three months and BMI>30 kg/m^2 (n=30)
c. For diabetic non-obese (GROUP NS-C): Type 2 diabetes diagnosis at least longer than 3 years; under stable medical treatment; HbA1c >7%; weight stability, defined as no significant change (5%) within the last three months and BMI<30 kg/m^2 (n=30)
d. For obese non-diabetics (GROUP NS-D): HbA1c<5.7%, No signs and history of T2D, and BMI>30 kg/m^2 (n=30)

1.3 Exclusion criteria:

a. Anti insulin / islet antibody and glutamic acid decarboxylase antibody (antiGAD) positivity, plasma fasting C-peptide lesser than 1 ng/ml.
b. Liver cirrhosis, severe renal failure, collagen diseases, severe endocrinopathies, blindness.
c. Heart failure, acute myocardial infarction, stroke or transient ischemic attack, unstable angina pectoris.
d. History of malignancy or malignant neoplasm in place, severe inflammatory complications, neurological or cardiovascular in act.
e. Pregnancy
f. Any conditions that at the discretion of the head of the study can represent risk to the patient or could affect the protocol results.

1.4 Recruitment of the subjects
The subjects for this non-surgical arm of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility. Patients who accept to stop their anti-diabetic medications 2 days prior to evaluation will be enrolled in the study.

2. Surgical Group:

2.1 Identification of study population

In the Surgical Group there are 4 different types of surgery. Age and sex matched patients who underwent laparoscopic sleeve gastrectomy (Group SG), mini-gastric bypass (Group MGB), sleeve gastrectomy with ileal transposition (Group IT) and sleeve gastrectomy with transit bipartition (Group TB) operated not less than 6 months, but within the last 2 years will be enrolled. The primary methodology of the study is to achieve adequate number of patients via an announcement through the website of Turkish Metabolic Surgery Foundation and divide the patients due to the mentioned categories.

In the surgery groups, we expect a change of the hormones by proximalising an intestinal limb and as a consequence activating the entero-insular axis. Similarly as observed in morbidly obese subjects after bariatric surgery, changes will be stable in the long time. Based in observations in morbidly obese and T2DM morbidly obese subjects we expect great reduction or disappearance of insulin resistance (IR) and improvement of beta-cell function, represented here by parameters obtained from mathematical model applied on MMT data (Fasting insulin secretion, Total insulin secretion, beta-cell glucose sensitivity, rate sensitivity and potentiation factor) with consecutive improvement of clinical T2DM symptoms and of the others components of the metabolic syndrome.

To assess the characteristics of distal ileal hormones we will perform an oral mixed meal tolerance test (OMTT), and analyze the parameters in Box 1. The exclusion criteria are the same as in the Non-surgical group.

2.2 Inclusion criteria

a. Type 2 Diabetic patients who underwent a sleeve gastrectomy, a mini-gastric bypass, a sleeve gastrectomy with ileal transposition or a sleeve gastrectomy with transit bipartition performed more than 6 months ago, but within the last 2 years, with steady weight profile (weight stability is defined as no significant change (5%) within the last three months)
b. Preferably not on any kind of anti-diabetic drugs or will accept cessation of all anti-diabetic drugs 2 days prior to evaluation.
    i. With either a reduction in HbA1c (compared to preoperative value) and/or reduction in insulin and/or reduction in antidiabetic drugs.
c. Absence of or resolved co-morbidities (dyslipidemia, hypertension, neuropathy, retinopathy, cardiovascular disease, stroke events or lower extremity amputation).
d. Possibility to participate to the quadruplicate measurement protocol.

2.3 Recruitment of the subjects

The subjects for this non-surgical arm of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility. Patients who accept to stop their anti-diabetic medications 2 days prior to evaluation will be enrolled in the study.

3. Intervention

Oral Mixed Meal Tolerance Test (OMTT): A standard mixed meal tolerance test (350 kcal, consisting of 55% carbohydrate, 25% protein, and 20% fat) is going to be performed in each participant. Venous blood samples will be collected at fasting stage and at 30, 60 and 120

minutes after the OMTT via catheter localized in ante-cubital vein. All blood samples will be drawn according to OMTT protocol (See Table 2).

4. Analytical Procedures

All the blood samples will be collected according to aforementioned OMTT protocol. Blood samples collected into ice-chilled tubes containing K2EDTA (spray-dried) tubes treated with DPP-4 inhibitor (BD Cat No: 366473 vacutainer® P700) will be used for PYY and, GLP-1 determinations. The tubes will be kept on ice until centrifuged in +4^{o}C for 20 min at 4000 g. Plasma will be separated and kept frozen at -20^{o}C immediately in aliquots of 30 ml until analysis. Serum separated by gel containing yellow levander tubes for the analysis of SGOT, SGPT, GGT, and whole blood samples collected into EDTA Na2 for HbA1c analysis are going to be used. Plasma glucose will be monitored from the plasma obtained from Fluoro-oxalate tubes (grey levander). Plasma insulin will be measured from plasma obtained from EDTA Na2 tubes. Liver function tests (SGOT, SGPT, and GGT) and HbA1c will have a single measurement during fasting. Plasma insulin levels and plasma glucose levels will be measured during fasting and 30-60-120 minutes after OMTT.

5. Outcomes Measured

During the visit a complete medical history and physical exam will be performed. Body weight, waist and hip circumference and Body Mass Index will be measured and recorded. The following outcomes (Box 1) will also be measured:

a. Plasma PYY will be measured by commercial ELISA kit of Biovender Research and Diagnostics products "Human PYY ELISA" Cat No: RSCYK080R with a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. The EIA kit shows 100% cross reactivity to human PYY (3-36) and human PYY (1-36), and shows less than 0.003% cross reactivity to human and rat NPY, which have similar amino acid sequence with human PYY.

Test Principle: This EIA kit for determination of human PYY in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. To the wells of plate coated with rabbit anti human PYY antibody, standard or samples, labeled antigen are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled streptoavidin-biotinylated antigen-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3'.

b. Plasma total GLP-1 will be measured by commercial ELISA kit of DRG® "GLP-1 (total) (EIA-5095) with a two-site "sandwich" technique with two selected GLP-1 antibodies.

Test Principle: This ELISA is designed, developed and produced for the quantitative measurement of GLP-1 (7-36) and (9-36) in plasma sample. The assay utilizes the two-site "sandwich" technique with two selected GLP-1 antibodies. Assay standards, controls and test samples are directly added to wells of a microplate that is coated with streptavidin. Subsequently, a mixture of biotinylated GLP-1 specific antibody and a horseradish peroxidate (HRP) conjugated GLP-1 specific antibody is added to each well. After the first incubation period, a "sandwich" immunocomplex of "Streptavidin – Biotin-Antibody – GLP-1(7-36)/(9-36) – HRP conjugated antibody" is formed and attached to the wall of the plate. The unbound HRP conjugated antibody is removed in a subsequent washing step. For the detection of this immunocomplex, each well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the

immunocomplex bound to GLP-1 (7-36)/(9-36) on the wall of the microtiter well is directly proportional to the amount of Total GLP-1 in the sample.

i. Sensitivity The sensitivity of this Total GLP-1 ELISA as determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.6 pmol/L.
ii. Specificity This Bioactive GLP-1 (7-36) assay is specific measure GLP-1 (7-36). It is expected that this assay does not detect following peptides.

1. GLP-1 (7-36) 100%
2. GLP-1 (9-36) 100%
3. GLP-1 (9-37) < 0.1%
4. GLP-1 (7-37) < 0.1%
5. GLP-1 (1-36) < 0.1%
6. GLP-2 < 0.1%
7. Glucagon < 0.1%

c. Liver Function Tests: Liver function tests (SGOT, SGPT, and GGT) will be measured by IFCC Enzymatic Assay in a Cobas 6000, Roche Diagnostics.

d. HbA1c: HbA1c will be measured by the turbidometric assay of Tina-quant Hemoglobin A1c Gen3 in a Cobas 6000 based on measurement of antipolyhapten complex.

e. Plasma Insulin Levels: will be measured by the ECLIA of Insulin Cobas 6000 based on sandwich assay. For quality control, PreciControl Multimarker or PreciControl Universal are going to be used.

f. Plasma Glucose Levels: will be measured by Enzymatic reference method with hexokinase. Hexokinase catalyzes the phosphorylation of glucose to glucose 6 phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrical.

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H+}$$

6. Primary Endpoints

The primary endpoint as the key outcome measure of the study will be area under the GLP-1, Peptide – YY, glucose and insulin curves following the OMTT.

7. Secondary Endpoints

Secondary study endpoints include: examination of the difference in the plasma levels of the distal ileal hormones in subjects with various health statuses and in patients who have been treated by different surgical techniques.

8. Ethics and informed consent

An independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board approved the study protocol. Oral and written informed consent from the patient will be obtained prior to inclusion.

9. Adverse Events

Although the Oral Mixed Meal Tolerance Test (OMTT) is considered safe, serious adverse events possibly related to the OMTT will be reported to the ethical committee

Ethics and dissemination

We have obtained ethical approval from an independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board. Oral and written informed consent from the patient will be obtained prior to inclusion. This study will take place in the Metabolic Surgery Clinic, Sisli, Istanbul, Turkey and the inclusion of patients will take place between Oktober 2015 and March 2016.
The subjects for the non-surgical group of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility.
The subjects for the surgical group there are 4 different types of surgery. Age and sex matched patients who underwent laparoscopic sleeve gastrectomy (Group SG), mini-gastric bypass (Group MGB), sleeve gastrectomy with ileal transposition (Group IT) and sleeve gastrectomy with transit bipartition (Group TB) operated not less than 6 months, but within the last 2 years will be enrolled. Via an announcement through the website of Turkish Metabolic Surgery Foundation patients will be recruited and divided in the above mentioned.
Educated lab personnel will do the OMTT, as well as the measurements of PYY and GLP-1.

The research nurses will collect the necessary data (from patient charts) and will store them on a secure harddrive. These harddrives will be collected and stored at the Metabolic Surgery Clinic in Istanbul in Turkey.

Dissemination plan

We expect that the results of this study, which will help us to understand the physiology regarding the enteric gut hormones and the improvement of glucose metabolism and insulin sensitivity after bariatric and metabolic surgery. The results will also give us insight in how to select patients for the specific bariatric surgical procedures. We expect the study to have some international appeal because of the increasing interest in gut hormone physiology and its correlation with patient outcomes (in terms of improvement/remission of type 2 diabetes after surgery). For end-of-study knowledge dissemination we intend to publish in medical, health services and/or public health journals. More importantly, we plan to present and discuss the results of our study on national and international congresses, focussing on surgery and endocrinology.

DISCUSSION

In this study we have three main hypotheses; 1) There will be increasing levels of GLP-1 and Peptide YY responses to a OMTT in individuals across diabetes-obesity spectrum; from those who are obese diabetics followed by non-obese diabetics, obese non-diabetic and healthy non-obese non-diabetics, 2) There will be increasing levels of GLP-1 and Peptide YY following a MMT in patients who have undergone a SG followed by MGB, SIT and STB, and 3) There will be marked improvements in glycemic control and insulin activity in techniques with bowel anastomosis, compared to SG. To our knowledge, this is the first study that aims to extensively research the glucose metabolism and secretion of ileal L-cell peptides in different metabolic states and surgical models.

There is increasing evidence that gut hormones play an important role in the neuro-endocrine physiology of hunger and satiety. As pointed out by Santoro et al. [21] and Celik et al. [19 20] there is need for a change in the current practice of bariatric/metabolic surgery. For these changes we have to focus on functional restriction, the proximal and distal gut imbalance and the role of gut hormones like, PYY and GLP-1 [19 21].

One of the important hormones in the proximal gut is GIP (glucose-dependent insulinotropic polypeptide). It is known as a counteractive hormone that produces an insulinic response, but instead of decreasing the secretion of glucagon, it enhances it [21]. In obese and diabetic patients there are abnormally high levels of GIP present (mainly a proximal gut product) [22]. Any kind of dietary restriction will lead to significant decreases in the GIP levels [23]. GIP is a hormone that is obesogenic and insulinotropic and strategies to block GIP production are beneficial for these patients [24 25].

The opposite, the distal gut hormones (e.g. GLP-1 and PYY) or their agonists are beneficial for obese and diabetics as well. Either way, blocking the hormonal activity of the proximal gut and increasing the activity of the distal gut, is beneficial. Surgical procedures support these findings [20]. Because of the sparse literature on the production of earlier mentioned hormones in different metabolic states and surgical models, a total of eight groups will be compared with each other (see table 1), to gain more insight in the physiology of glucose and hormonal metabolism.

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A Celik has nothing to disclose

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TABLE 1: Overview of study groups and outcomes measured

NON-SURGICAL GROUPS (n=120)

1. Healthy volunteers (n=30)
2. Obese diabetics (n=30)
3. Obese non-diabetics (n=30)
4. Non-obese diabetics (n=30)

SURGERY GROUPS (n=120)

5. Sleeve Gastrectomy (SG, n=30)
6. Mini Gastric Bypass (MGB, n=30)
7. Sleeve Gastrectomy with Ileal Transposition (IT, n=30)
8. Sleeve Gastrectomy with Transit Bipartition (TB, n=30)

Box 1: Outcomes Measured:

* Baseline and 30-60-120 minutes GLP-1, and Peptide YY response to an OMTT,
* Baseline and 30-60-120 minutes plasma insulin and glucose measurements,
* Fasting lipid profile: total cholesterol, HDL and LDL-cholesterol and triglycerides,
* Liver profile: AST, ALT and GGT,
* Body weight, BMI, waist and hip circumference

TABLE 2: Oral Mixed Meal Tolerance Test (OMTT) Protocol

	Yellow levander with gel separator	Na2 EDTA	2 X K2 EDTA +DPP IV inhibitor	
Time				**Volume**
0	**7**	**3**	**6**	**16**
30	**7**	**3**	**6**	**16**
60	**7**	**3**	**6**	**16**
120	7	3	6	**16**

- **Yellow Levander with Gel Separator:** SGOT, SGPT, GGT (all in u/L)
- **Na2EDTA:** = HbA1c (mmol/mol)
- **2xK2EDTA + DPP IV inhibitor:** Glucagon Like Peptide-1 (GLP-1 in pmol/l); Peptide YY (in pg/ml)

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Effects of different metabolic states and surgical models on glucose metabolism and secretion of ileal L-cell peptides: a study protocol for a cross-sectional study

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Effects of different metabolic states and surgical models on glucose metabolism and secretion of ileal L-cell peptides: a study protocol for a cross-sectional study

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ABSTRACT

Introduction: Obesity and type 2 diabetes mellitus (T2DM) are increasing worldwide, thus reaching pandemic proportions. The role functional restriction and gut hormones can be a beneficial tool in treating obesity and diabetes. However, the exact hormonal profiles in different metabolic states and surgical models are unknown.

Methods and analysis: The HIPER-1 study is a single centre cross sectional study in which a total 240 patients (in different metabolic states and surgical models) will receive an Oral Mixed Meal Tolerance Test (OMTT). At baseline and after 30, 60 and 120 minutes the PYY levels, GLP-1 levels, glucose and insulin sensitivity will be measured. The primary endpoint of the study will be the area under the GLP-1 and Peptide – YY curves following the OMTT. Secondary study endpoints include: examination of the difference in the plasma levels of the distal ileal hormones in subjects with various health statuses and in patients who have been treated with different surgical techniques.

Ethics and dissemination An independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board approved the study protocol. Dissemination will occur via publication, national and international conference presentations, and exchanges with regional, provincial and national stakeholders.

Trial registration number: NCT02532829

Strengths and limitations of this study

Strengths:

- The HIPER-1 study gives new insight in gut hormone profiles in different metabolic states and surgical models
- The HIPER-1 study gives insight in glucose metabolism and insulin resistance and its correlation with the gut hormones

Limitations:

- This study is limited to four surgical procedures: sleeve gastrectomy, mini gastric bypass, sleeve gastrectomy with ileal transposition and sleeve gastrectomy with transit bipartition.
- This is a single centre study performed in a Turkish metabolic surgery clinic with a specific patient population, which may give problems in terms of generalisability of the study results.
- Utilisation of only a 2-day washout period for diabetes medication.

INTRODUCTION

Obesity and type 2 diabetes mellitus (T2DM) are increasing worldwide, reaching pandemic proportions [1]. Diet, exercise and medication remain the cornerstones for the treatment of T2DM. But, apart from studies demonstrating promising results in some of the developed countries; the long-term success rates of lifestyle and drug modifications are disappointing [2]. Even with an impressive armamentarium of medication, adequate long-term glycemic control is difficult, and overly tight glycemic control introduces a proportionate risk of hypoglycemia and targets have been modified because of the high risk of cardiovascular events [3]. Moreover, diabetes medication can promote weight gain, which in turn exacerbates the obesity issues [4 5]. In cases where classic surgical strategies proved to be inadequate, broad type of gastrointestinal (GI) surgical methods offer new alternatives to treat obesity and T2DM [6]. Among severely obese patients, bariatric surgical options cause significant sustained weight loss, improvement of obesity-related co-morbidities, and reduction in long-term mortality [7]. Currently, bariatric surgery is considered to be appropriate for individuals with a body mass index (BMI) >35 kg/m^2 and serious obesity-related comorbidities, including T2DM. Surgical procedures involving intestinal bypasses exert particularly higher effects on diabetes [8]. Mounting evidence indicates that these remarkable effects result not only from weight loss but also from weight-independent anti-diabetic mechanisms [9]. Consequently, conventional bariatric procedures and new experimental GI operations are being explored for the management of patients with T2DM and are overweight or class I obese (BMI: 30-35 kg/m^2).

Many physiological mechanisms proposed to explain the improvement of glucose metabolism, insulin metabolism and beta-cell function following surgery include:

a) Major diet restriction early after surgery,
b) Hepatic insulin sensitivity recovery early after surgery,
c) Increase of incretin hormone (GLP-1) caused by rearrangements in the gastro-intestinal tract,
d) The earlier blockage of glucagon secretion, caused by GLP-1,
e) Less hunger and early satiety (changes in Ghrelin, GLP-1, PYY and Oxyntomodulin (OXM)
f) Recovery of Beta-cell function by incretins stimulation and
g) Weight loss induced reduction in beta-cell gluco- and lipotoxicity [10-14].

Evidence gaps to be filled

The complete mechanisms of glucose metabolism, insulin metabolism and the changes after metabolic surgery remain poorly understood. The variable levels of incretin stimulation (especially GLP-1) and improved glycaemic control in those with diabetes have been shown following various bariatric techniques [10-13]. PYY1-36 is also synthesized and released from specialized entero-endocrine cells called L-cells found predominantly within the distal GI tract (hindgut) and is then cleaved by the enzyme DPP-4 to give the active form, PYY3-36 [15]. In our study, we will measure the active PYY3-36 to better document the effects of active form of PYY for 8 groups. The measurement of serum PYY in No Surgery and Surgery Groups will give us the pattern of PYY stimulation in different groups. Since all 8 groups will be tested with the standard Oral Mixed Meal Tolerance Test (OMTT) the macronutrients effect on PYY peak will be overcome [16]. “Ileal brake” term can be considered as a summary of GLP-1 and PYY actions on gut including reduction in gastric emptying and delay in intestinal transit, which can be used as a good tool for the treatment of people with obesity and related conditions. We must add that they also act on both peripheral and central nervous systems concentrating at the arcuate nucleus of the hypothalamus (ARC), which plays a key role in the regulation of appetite. Batterham et al. [17] published its effects on rats and next year documented the PYY effects on humans [18] indicating that the basal levels of PYY in obese

subjects when compared with normal weight subjects were lower. There was also a blunted postprandial PYY rise suggesting that a lack of endogenous PYY secretion may be implicated in the development of obesity. In our study, we expect to see different patterns of serum PYY levels to better explain its role in the 8 different groups. GLP-1 is secreted from distal intestinal L-cells along with Peptide YY and Oxyntomodulin in response to a meal. But little is known about the levels of distal intestinal L-cell hormones in healthy individuals, different disease states, and different body compositions. Also, the difference about the baseline values and activities of these hormones after different surgical techniques has not been extensively studied. The present study will give insight in the physiology of these gut hormones and its relation to the glucose metabolism after metabolic surgery. Secondly this study will give insight in differences between gut hormone levels in different metabolic states and after different surgical procedures, which will be necessary in understanding physiological aspects of these gut hormones.

SPECIFIC AIMS:
We plan to test our hypothesis and, thereby accomplish the objective of this application by pursuing the following specific aims:

Aim 1: To measure and compare the levels of GLP-1 and Peptide YY in non-obese healthy volunteers vs. obese diabetics vs. obese non-diabetics vs. non-obese diabetics with administration of a standardized OMTT at baseline, 30-60-120 minutes.
Hypothesis 1: There will be increasing levels of GLP-1 and Peptide YY responses to a OMTT in individuals across diabetes-obesity spectrum; from those who are obese diabetics followed by non-obese diabetics, obese non-diabetic and healthy non-obese non-diabetics.

Aim 2: To measure and compare the levels of GLP-1 and Peptide YY in patients who have undergone sleeve gastrectomy (SG) vs. Mini-gastric bypass (MGB) vs. Sleeve gastrectomy with Ileal Transposition (SIT) vs. Sleeve gastrectomy with Transit Bipartition (STB) after administration of OMTT at baseline, 30-60-120 minutes.
Hypothesis 2: There will be increasing levels of GLP-1 and Peptide YY following an OMMT in patients who have undergone a SG followed by MGB, SIT and STB.

Aim 3: To analyze the response of insulin and glucose following the OMTT in relation to the type of surgery.
Hypothesis 3: There will be marked improvements in glycaemic control and insulin activity in techniques with bowel anastomosis, compared to SG.

In this study, we aimed to analyse the baseline levels and 30-60-120 min postprandial activities of GLP-1, and Peptide YY in No Surgery and Surgery Groups (defined below). This will be the initial evaluation of a durability study that is planned for a minimum of 5 years follow up.

METHODS
This cross-sectional study will be performed at the Metabolic Surgery Clinic in Istanbul. Inclusion will be performed by the physician researcher after written informed consent. The study consists of a non-surgical and a surgical group.

Sample size calculation and statistical analysis
The study is fashioned as a cross-sectional analysis and it will be an IRB approved prospective study. Sample size calculation is based on the formula of Kelsey. The sample size

will be 120 subjects in the surgical group and 120 subjects in the non-surgical group (which means 30 subjects in each subgroup) based on a significance level of 5%, a power of 80% and a mean decrease of fasting glucose level (postoperative) of 35%. [19 20]
In total of 240 patients will be included in this study (table 1). Continuous variables will be presented as mean ± standard deviation (SD). Categorical variables were presented as frequency with percentages. Statistical analysis will be performed by Repeated Measurement Analysis of Variance and 1-way ANOVA.

In all tests, values of $p<0.05$ were considered statistically significant. Statistical Package for Social Sciences (SPSS, Chicago, IL, USA Version 20.0) will be used to prepare the database and for statistical analysis.

1. Non-Surgical Group:

1.1 Study population
We will be studying 120 subjects (aged 30 to 60 years) in the non-surgical groups.

1.2 Inclusion criteria:

a. For healthy subjects (GROUP NS-A): No known disease, no previous surgery, HbA1c<5.7%, BMI<25 kg/m^2 (n=30)
b. For diabetic obese (GROUP NS-B): Type 2 diabetes diagnosis at least longer than 3 years; under stable medical treatment (no changes in medication or insulin dosage have been made in the last 6 months); HbA1c >7%; weight stability, defined as no significant change (5%) within the last three months and BMI>30 kg/m^2 (n=30)
c. For diabetic non-obese (GROUP NS-C): Type 2 diabetes diagnosis at least longer than 3 years; under stable medical treatment (no changes in medication or insulin dosage have been made in the last 6 months); HbA1c >7%; weight stability, defined as no significant change (5%) within the last three months and BMI<30 kg/m^2 (n=30)
d. For obese non-diabetics (GROUP NS-D): HbA1c<5.7%, No signs and history of T2D, and BMI>30 kg/m^2 (n=30)

1.3 Exclusion criteria:

a. Anti insulin / islet antibody and glutamic acid decarboxylase antibody (antiGAD) positivity, plasma fasting C-peptide lesser than 1 ng/ml.
b. Liver cirrhosis, severe renal failure, collagen diseases, severe endocrinopathies, blindness.
c. Heart failure, acute myocardial infarction, stroke or transient ischemic attack, unstable angina pectoris.
d. History of malignancy or malignant neoplasm in place, severe inflammatory complications, neurological or cardiovascular in act.
e. Pregnancy
f. Any conditions that at the discretion of the head of the study can represent risk to the patient or could affect the protocol results.

1.4 Recruitment of the subjects
The subjects for this non-surgical arm of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study

personnel who will provide study related information and screen the patient for initial eligibility. Patients who accept to stop their anti-diabetic medications 2 days prior to evaluation will be enrolled in the study.

2. Surgical Group:

2.1 Identification of study population

In the Surgical Group there are 4 different types of surgery. Age and sex matched patients who underwent laparoscopic sleeve gastrectomy (Group SG), mini-gastric bypass (Group MGB), sleeve gastrectomy with ileal transposition (Group IT) and sleeve gastrectomy with transit bipartition (Group TB) operated not less than 6 months, but within the last 2 years will be enrolled. The primary methodology of the study is to achieve adequate number of patients via an announcement through the website of Turkish Metabolic Surgery Foundation and divide the patients due to the mentioned categories.

In the surgery groups, we expect a change of the hormones by proximalising an intestinal limb and as a consequence activating the entero-insular axis. Similarly as observed in morbidly obese subjects after bariatric surgery, changes will be stable in the long time. Based in observations in morbidly obese and T2DM morbidly obese subjects we expect great reduction or disappearance of insulin resistance (IR) and improvement of beta-cell function, represented here by parameters obtained from mathematical model applied on MMT data (Fasting insulin secretion, Total insulin secretion, beta-cell glucose sensitivity, rate sensitivity and potentiation factor) with consecutive improvement of clinical T2DM symptoms and of the others components of the metabolic syndrome.

To assess the characteristics of distal ileal hormones we will perform an oral mixed meal tolerance test (OMTT), and analyze the parameters in Box 1. The exclusion criteria are the same as in the Non-surgical group.

2.2 Inclusion criteria

a. Type 2 Diabetic patients who underwent a sleeve gastrectomy, a mini-gastric bypass, a sleeve gastrectomy with ileal transposition or a sleeve gastrectomy with transit bipartition performed more than 6 months ago, but within the last 2 years, with steady weight profile (weight stability is defined as no significant change (5%) within the last three months)
b. Preferably not on any kind of anti-diabetic drugs or will accept cessation of all anti-diabetic drugs 2 days prior to evaluation.
    i. With either a reduction in HbA1c (compared to preoperative value) and/or reduction in insulin and/or reduction in antidiabetic drugs.
c. Absence of or resolved co-morbidities (dyslipidemia, hypertension, neuropathy, retinopathy, cardiovascular disease, stroke events or lower extremity amputation).
d. Possibility to participate to the quadruplicate measurement protocol.

2.3 Recruitment of the subjects

The subjects for this non-surgical arm of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility. Patients who accept to stop their anti-diabetic medications 2 days prior to evaluation will be enrolled in the study.

3. Intervention

Oral Mixed Meal Tolerance Test (OMTT): A standard mixed meal tolerance test (350 kcal, consisting of 55% carbohydrate, 25% protein, and 20% fat) is going to be performed in each participant. Venous blood samples will be collected at fasting stage and at 30, 60 and 120

minutes after the OMTT via catheter localized in ante-cubital vein. All blood samples will be drawn according to OMTT protocol (See Table 2).

4. Analytical Procedures

All the blood samples will be collected according to aforementioned OMTT protocol. Blood samples collected into ice-chilled tubes containing K2EDTA (spray-dried) tubes treated with DPP-4 inhibitor (BD Cat No: 366473 vacutainer® P700) will be used for PYY and, GLP-1 determinations. The tubes will be kept on ice until centrifuged in +4^{o}C for 20 min at 4000 g. Plasma will be separated and kept frozen at -20^{o}C immediately in aliquots of 30 ml until analysis. Serum separated by gel containing yellow levander tubes for the analysis of SGOT, SGPT, GGT, and whole blood samples collected into EDTA Na2 for HbA1c analysis are going to be used. Plasma glucose will be monitored from the plasma obtained from Fluoro-oxalate tubes (grey levander). Plasma insulin will be measured from plasma obtained from EDTA Na2 tubes. Liver function tests (SGOT, SGPT, and GGT) and HbA1c will have a single measurement during fasting. Plasma insulin levels and plasma glucose levels will be measured during fasting and 30-60-120 minutes after OMTT.

5. Outcomes Measured

During the visit a complete medical history and physical exam will be performed. Body weight, waist and hip circumference and Body Mass Index will be measured and recorded. The following outcomes (Box 1) will also be measured:

a. Plasma PYY will be measured by commercial ELISA kit of Biovender Research and Diagnostics products "Human PYY ELISA" Cat No: RSCYK080R with a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. The EIA kit shows 100% cross reactivity to human PYY (3-36) and human PYY (1-36), and shows less than 0.003% cross reactivity to human and rat NPY, which have similar amino acid sequence with human PYY.

Test Principle: This EIA kit for determination of human PYY in samples is based on a competitive enzyme immunoassay using combination of highly specific antibody to human PYY and biotin-avidin affinity system. To the wells of plate coated with rabbit anti human PYY antibody, standard or samples, labeled antigen are added for competitive immunoreaction. After incubation and plate washing, horse radish peroxidase (HRP) labeled streptoavidin (SA) is added to form HRP labeled streptoavidin-biotinylated antigen-antibody complex on the surface of the wells. Finally, HRP enzyme activity is determined by 3,3'.

b. Plasma total GLP-1 will be measured by commercial ELISA kit of DRG® "GLP-1 (total) (EIA-5095) with a two-site "sandwich" technique with two selected GLP-1 antibodies. *Test Principle*: This ELISA is designed, developed and produced for the quantitative measurement of GLP-1 (7-36) and (9-36) in plasma sample. The assay utilizes the two-site "sandwich" technique with two selected GLP-1 antibodies. Assay standards, controls and test samples are directly added to wells of a microplate that is coated with streptavidin. Subsequently, a mixture of biotinylated GLP-1 specific antibody and a horseradish peroxidate (HRP) conjugated GLP-1 specific antibody is added to each well. After the first incubation period, a "sandwich" immunocomplex of "Streptavidin – Biotin-Antibody – GLP-1(7-36)/(9-36) – HRP conjugated antibody" is formed and attached to the wall of the plate. The unbound HRP conjugated antibody is removed in a subsequent washing step. For the detection of this immunocomplex, each well is then incubated with a substrate solution in a timed reaction and then measured in a spectrophotometric microplate reader. The enzymatic activity of the

immunocomplex bound to GLP-1 (7-36)/(9-36) on the wall of the microtiter well is directly proportional to the amount of Total GLP-1 in the sample.

i. Sensitivity The sensitivity of this Total GLP-1 ELISA as determined by 3 times the standard deviation above zero standard on 12 replicate determinations is approximately 0.6 pmol/L.
ii. Specificity This Bioactive GLP-1 (7-36) assay is specific measure GLP-1 (7-36). It is expected that this assay does not detect following peptides.

1. GLP-1 (7-36) 100%
2. GLP-1 (9-36) 100%
3. GLP-1 (9-37) < 0.1%
4. GLP-1 (7-37) < 0.1%
5. GLP-1 (1-36) < 0.1%
6. GLP-2 < 0.1%
7. Glucagon < 0.1%

c. Liver Function Tests: Liver function tests (SGOT, SGPT, and GGT) will be measured by IFCC Enzymatic Assay in a Cobas 6000, Roche Diagnostics.

d. HbA1c: HbA1c will be measured by the turbidometric assay of Tina-quant Hemoglobin A1c Gen3 in a Cobas 6000 based on measurement of antipolyhapten complex.

e. Plasma Insulin Levels: will be measured by the ECLIA of Insulin Cobas 6000 based on sandwich assay. For quality control, PreciControl Multimarker or PreciControl Universal are going to be used.

f. Plasma Glucose Levels: will be measured by Enzymatic reference method with hexokinase. Hexokinase catalyzes the phosphorylation of glucose to glucose 6 phosphate by ATP.

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{G-6-P} + \text{ADP}$$

Glucose-6-phosphate dehydrogenase oxidizes glucose-6-phosphate in the presence of NADP to gluconate-6-phosphate. No other carbohydrate is oxidized. The rate of NADPH formation during the reaction is directly proportional to the glucose concentration and is measured photometrical.

$$\text{G-6-P} + \text{NADP}^{+} \xrightarrow{\text{G-6-PDH}} \text{gluconate-6-P} + \text{NADPH} + \text{H+}$$

6. Primary Endpoints

The primary endpoint as the key outcome measure of the study will be area under the GLP-1, Peptide – YY, glucose and insulin curves following the OMTT.

7. Secondary Endpoints

Secondary study endpoints include: examination of the difference in the plasma levels of the distal ileal hormones in subjects with various health statuses and in patients who have been treated by different surgical techniques.

8. Ethics and informed consent

An independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board approved the study protocol. Oral and written informed consent from the patient will be obtained prior to inclusion.

9. Adverse Events

Although the Oral Mixed Meal Tolerance Test (OMTT) is considered safe, serious adverse events possibly related to the OMTT will be reported to the ethical committee

Ethics and dissemination

We have obtained ethical approval from an independent ethics committee, the Istanbul Sisli Kolan International Hospital, Turkey Institutional Review Board. Oral and written informed consent from the patient will be obtained prior to inclusion. This study will take place in the Metabolic Surgery Clinic, Sisli, Istanbul, Turkey and the inclusion of patients will take place between Oktober 2015 and March 2016.
The subjects for the non-surgical group of the study will be recruited from the websites of surgical and medical associations and formally announced in the meetings. The study will also be promoted in national organizations. Interested candidates will be directed to study personnel who will provide study related information and screen the patient for initial eligibility.
The subjects for the surgical group there are 4 different types of surgery. Age and sex matched patients who underwent laparoscopic sleeve gastrectomy (Group SG), mini-gastric bypass (Group MGB), sleeve gastrectomy with ileal transposition (Group IT) and sleeve gastrectomy with transit bipartition (Group TB) operated not less than 6 months, but within the last 2 years will be enrolled. Via an announcement through the website of Turkish Metabolic Surgery Foundation patients will be recruited and divided in the above mentioned.
Educated lab personnel will do the OMTT, as well as the measurements of PYY and GLP-1.

The research nurses will collect the necessary data (from patient charts) and will store them on a secure harddrive. These harddrives will be collected and stored at the Metabolic Surgery Clinic in Istanbul in Turkey.

Dissemination plan

We expect that the results of this study, which will help us to understand the physiology regarding the enteric gut hormones and the improvement of glucose metabolism and insulin sensitivity after bariatric and metabolic surgery. The results will also give us insight in how to select patients for the specific bariatric surgical procedures. We expect the study to have some international appeal because of the increasing interest in gut hormone physiology and its correlation with patient outcomes (in terms of improvement/remission of type 2 diabetes after surgery). For end-of-study knowledge dissemination we intend to publish in medical, health services and/or public health journals. More importantly, we plan to present and discuss the results of our study on national and international congresses, focussing on surgery and endocrinology.

DISCUSSION

In this study we have three main hypotheses; 1) There will be increasing levels of GLP-1 and Peptide YY responses to a OMTT in individuals across diabetes-obesity spectrum; from those who are obese diabetics followed by non-obese diabetics, obese non-diabetic and healthy non-obese non-diabetics, 2) There will be increasing levels of GLP-1 and Peptide YY following a MMT in patients who have undergone a SG followed by MGB, SIT and STB, and 3) There will be marked improvements in glycemic control and insulin activity in techniques with bowel anastomosis, compared to SG. To our knowledge, this is the first study that aims to extensively research the glucose metabolism and secretion of ileal L-cell peptides in different metabolic states and surgical models.

There is increasing evidence that gut hormones play an important role in the neuro-endocrine physiology of hunger and satiety. As pointed out by Santoro et al. [21] and Celik et al. [19 20] there is need for a change in the current practice of bariatric/metabolic surgery. For these changes we have to focus on functional restriction, the proximal and distal gut imbalance and the role of gut hormones like, PYY and GLP-1 [19 21].

One of the important hormones in the proximal gut is GIP (glucose-dependent insulinotropic polypeptide). It is known as a counteractive hormone that produces an insulinic response, but instead of decreasing the secretion of glucagon, it enhances it [21]. In obese and diabetic patients there are abnormally high levels of GIP present (mainly a proximal gut product) [22]. Any kind of dietary restriction will lead to significant decreases in the GIP levels [23]. GIP is a hormone that is obesogenic and insulinotropic and strategies to block GIP production are beneficial for these patients [24 25].

The opposite, the distal gut hormones (e.g. GLP-1 and PYY) or their agonists are beneficial for obese and diabetics as well. Either way, blocking the hormonal activity of the proximal gut and increasing the activity of the distal gut, is beneficial. Surgical procedures support these findings [20]. Because of the sparse literature on the production of earlier mentioned hormones in different metabolic states and surgical models, a total of eight groups will be compared with each other (see table 1), to gain more insight in the physiology of glucose and hormonal metabolism.

Conflict of Interest:

A Celik has nothing to disclose

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S. Pouwels has nothing to disclose
B. Celik has nothing to disclose
F. Karaca has nothing to disclose
S. Santoro is on the Ethicon Advisory Board

A. Gupta has nothing to disclose
S. Ugale has nothing to disclose

TABLE 1: Overview of study groups and outcomes measured

NON-SURGICAL GROUPS (n=120)

1. Healthy volunteers (n=30)
2. Obese diabetics (n=30)
3. Obese non-diabetics (n=30)
4. Non-obese diabetics (n=30)

SURGERY GROUPS (n=120)

5. Sleeve Gastrectomy (SG, n=30)
6. Mini Gastric Bypass (MGB, n=30)
7. Sleeve Gastrectomy with Ileal Transposition (IT, n=30)
8. Sleeve Gastrectomy with Transit Bipartition (TB, n=30)

Box 1: Outcomes Measured:

* Baseline and 30-60-120 minutes GLP-1, and Peptide YY response to an OMTT,
* Baseline and 30-60-120 minutes plasma insulin and glucose measurements,
* Fasting lipid profile: total cholesterol, HDL and LDL-cholesterol and triglycerides,
* Liver profile: AST, ALT and GGT,
* Body weight, BMI, waist and hip circumference

TABLE 2: Oral Mixed Meal Tolerance Test (OMTT) Protocol

	Yellow levander with gel separator	Na2 EDTA	2 X K2 EDTA +DPP IV inhibitor	
Time				**Volume**
0	**7**	**3**	**6**	**16**
30	**7**	**3**	**6**	**16**
60	**7**	**3**	**6**	**16**
120	7	3	6	**16**

- **Yellow Levander with Gel Separator:** SGOT, SGPT, GGT (all in u/L)
- **Na2EDTA:** = HbA1c (mmol/mol)
- **2xK2EDTA + DPP IV inhibitor:** Glucagon Like Peptide-1 (GLP-1 in pmol/l); Peptide YY (in pg/ml)

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Sjaak Pouwels has nothing to disclose
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Fatih Karaca has nothing to disclose
Sergio Santoro is on the Ethicon Advisory Board

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