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PROTOCOL

MOMS protocol contains the following amendments, in a chronological/ethical approval sequence:

MOMS Protocol		Page #
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FINAL PROTOCOL

PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

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LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
ADA	American Diabetes Association
AMP	Adenosine monophosphate
Anti-GAD	Antibody anti-glutamic acid decarboxylase
CDC	Centers for Disease Control and Prevention
IRB	Internal Review Board
CONEP	National Research Ethics Committee
DCCT	Diabetes Control and Complications Trial
T2DM	Type 2 diabetes mellitus
DPP-IV	Dipeptidyl peptidase-IV
USA	United States of America
FDA	Food and Drug Administration
GLP-1	Glucagon-like peptide-1
GIP	Gastric inhibitory polypeptide
HbA1C	Glycosylated hemoglobin A1c
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
BMI	Body mass index
ISO	International Organization for Standardization
Kg/m^2	Kilograms per square meter
LDL	Low density protein
MDRD	Modification of diet in renal disease
SF-36	Health-related and general quality of life survey (Short form-36)
OABq-SF	Overactive Bladder Questionaire Short Form
CRP	C Reative Protein
UKPDS	United Kingdom Prospective Study
IPPS	International Prostate Symptoms Score

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1. INTRODUCTION

T2DM is a heterogeneous disorder characterized by defects in insulin secretion and sensitivity. Insulin resistance is the initial event in T2DM, and beta-cell function gradually declines until hyperglycemia is evident. Many mechanisms have been suggested as causes of insulin resistance, such as increase in non-esterified fatty acids, inflammatory cytokines and adipokines. In addition, endothelial and beta-cell dysfunction, glucotoxicity, lipotoxicity, defective incretin action (GLP-1 and GIP), and deposit of amyloid substance are also implicated in its physiopathology [STUMVOLL, 2005; NYENWE, 2011; DeFRONZO, 2009].

1.1 Pathogenesis of T2DM

Individuals prone to develop T2DM inherit certain genes involved in the development of insulin resistance, according to different tissues [DeFRONZO, 1988; ERIKSSON, 1989; PENDERGRASS, 2007; GROOP, 2008]. In the liver, one of the signs of insulin resistance is increased glucose production [DeFRONZO, 1989], whereas in the muscles, inhibited glucose uptake is evident, causing postprandial hyperglycemia [FERRANNINI, 1988]. In adipocytes, insulin resistance causes lipolysis and release of free fatty acids (lipotoxicity) [BAYS, 2004], with consequent deposition in the hepatocytes, myocytes, beta-cells and endothelium (accelerating atherosclerosis), which leads to increased production of inflammatory adipocytokines and worsening of insulin resistance [DeFRONZO, 2009].

Other mechanisms that may be involved in the pathogenesis of T2DM are hyperglucagonemia, produced by pancreatic alpha-cells and leading to enhanced hepatic glucose production (BARON, 1987], and increased renal glucose reabsorption [RAHMOUNE, 2005; DeFRONZO, 2009].

Although genetic mechanisms are involved in the development of insulin resistance, the T2DM epidemic is linked to the obesity epidemic [DeFRONZO, 1978] and sedentary lifestyle [KOIVISTO, 1986] of the Western population. Obesity and sedentary lifestyle, in combination with a genetic predisposition, lead to beta-cell overload, promoting a greater secretion of insulin in response to defective insulin action. Gradually, this overload results in beta-cell failure, which occurs when there is loss of approximately 80% of these cells' mass in the pancreas [WEYER, 2001; BUTLER, 2003]. Thus, these individuals develop fasting hyperglycemia, progressing to T2DM [DeFRONZO, 2009].

1.2 Incidence of T2DM and its Complications

The World Health Organization estimated that in 2000 there were approximately 170 million diabetics in the world. This figure is believed to double by 2030, reaching about 336 million individuals. In the Americas, the number of cases of type 2 diabetes will increase from 33 million to 66.8 million in the same period.

These patients' glycemic control is particularly difficult, as it has been shown in a study conducted in Brazil, in which 85% of patients had serum glycosylated hemoglobin

(HbA1c) level > 7.0%, which is frequently associated with microvascular complications such as nephropathy, retinopathy, and neuropathy [WILD, 2004; MENDES, 2010; REMUZZI, 2002; WATKINS, 2003; YOUNG, 1993].

In the United States, the number of people who started treatment for end-stage renal disease caused by diabetes increased from 2,600 in 1980 to 48,374 in 2008. In 2006, a survey conducted by the CDC found 66,000 nontraumatic lower limb amputations in diabetics in the United States. In addition, in 2009, 19.7% of U.S. diabetic adults had visual acuity impairment.

1.3 Clinical Treatment of T2DM

The United Kingdom Prospective Study (UKPDS) study showed that early intensive glycemic control reduces the risk of developing microvascular complications in T2DM patients in medium [UKPDS, 1998] and long terms [HOLMAN, 2008]. However, the Diabetes Control and Complications Trial (DCCT) reported a paradoxical worsening of microvascular complications, such as retinopathy and neuropathy, after faster glycemic control [DCCT, 1993]. The safety and efficacy of a rigorous glycemic control have been questioned in recent studies [PATEL, 2008; DUCKWORTH, 2009; GERSTEIN, 2008; BUCHWALD, 2009]. The concept of "metabolic memory" together with side effects of an intensive pharmacological treatment may explain the discrepancy of these studies' results.

Despite the development of new medications for T2DM, these drugs are equally or less effective than the three previous classes of drugs (insulin, biguanide, and sulfonylurea) to reduce blood glucose, as shown in the table below (Table 1). Furthermore, these new drugs are more expensive [NATHAN, 2007a]. In addition, with the evolution of the disease and because it is a multicausal disease, combinations of various classes of antidiabetic medications are necessary to achieve disease control. And most of the time such control is not achieved, even with so many pharmacological options [DeFRONZO, 2009; MENDES, 2010].

Nine classes of medications for T2DM have been approved so far by the FDA in the United States, as shown in the Table 1.

Table 1. Antidiabetic Drugs Approved in the United States

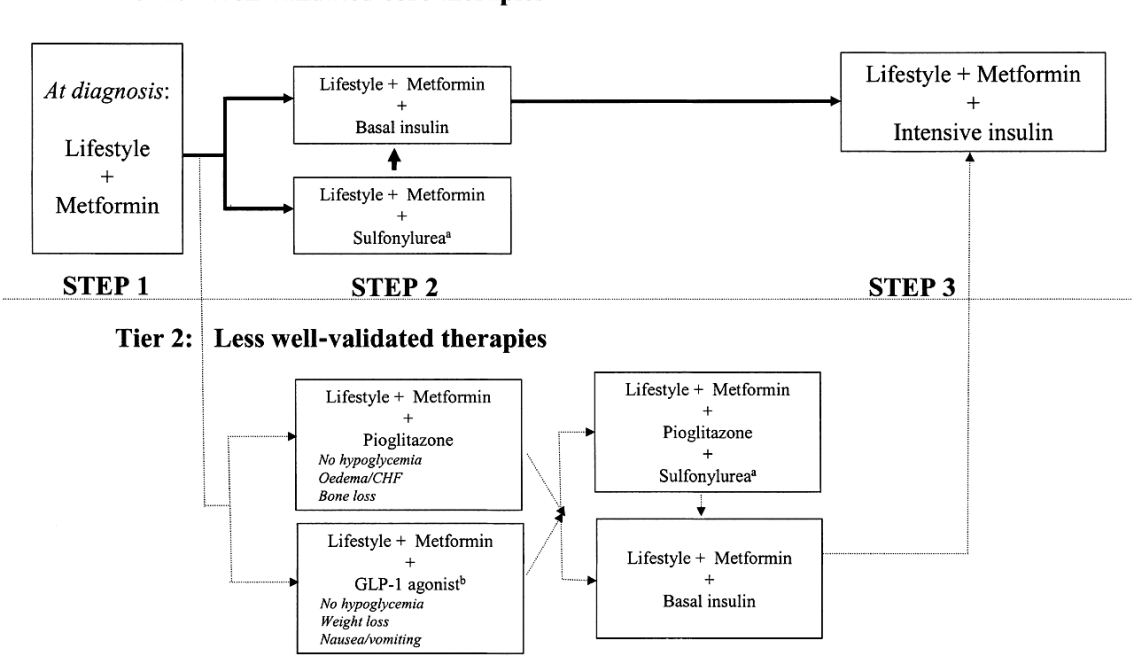
Drug	Route of Administration	Market Release Date	% of reduction in HbA1c when used as a monotherapy
Insulin	Parenteral	1921	≥2.5
Sulfonylurea	Oral	1946	1.5
Biguanide	Oral	1957	
Metformin	Oral	1995	1.5
Alpha-glucosidase inhibitors	Oral	1995	0.5-0.8

Thiazolidinedione	Oral		0.8-1.0
Pioglitazone	Oral	1999	
Glinide	Oral	1997	1.0-1.5
GLP analogs	Parenteral	2005	0.6
Amylin analogs	Parenteral	2005	0.6
DPP-IV inhibitors	Oral	2006	0.5-0.9

Adapted from NATHAN, 2007 b.

In 2009, a consensus algorithm for the initiation and adjustment of therapy was published in Diabetes Care (NATHAN, 2007 b), as shown below.

Fig. 1. Consensus algorithm for the initiation and adjustment of therapy in Diabetic Patients'



Despite these recommendations, DeFronzo suggests that the best clinical treatment for T2DM is a combined therapy acting against the causes of the disease and preserving the maximum mass of pancreatic beta-cells. In the liver, both metformin [CUSI, 1996] and thiazolidinedione [BAYS, 2004] are potent insulin sensitizers and inhibit the T2DM-typical increased gluconeogenesis. In the muscles, thiazolidinedione has the same insulin sensitization action [MIYAZAKI, 2001]. Whereas thiazolidinedione acts on the insulin signaling pathway, metformin acts on AMP kinase pathway, both leading to an additive effect in reducing HbA1c without causing hypoglycemia, since they do not affect insulin secretion. In the adipose tissue, thiazolidinedione has also an excellent insulin sensitization action, leading to inhibition of lipolysis, removing fat for from the muscles, liver, and beta-cells, improving lipotoxicity, enhancing and preserving beta-cell function [BAYS, 2004; DeFRONZO, 2009]. Although leading to weight gain and increased chance of hypoglycemia,

insulin initiation is necessary if oral medications were not sufficient to achieve HbA1c target levels.

There is evidence that GLP-1 analogs can also preserve beta-cell function [KLONOFF, 2008]. In addition, these analogs reduce hepatic glucose production in the liver, decrease glucagon secretion by pancreatic alpha-cells, reduce gastric emptying in the gastrointestinal tract by and resolve GLP-1 deficit, while reducing appetite and leading to weight loss. It is worth mentioning that the GLP-1 action of increasing insulin secretion dissipates when normoglycemia is achieved, preventing hypoglycemia. There is not enough evidence that inhibitors of DPP-IV, an enzyme that degrades incretins (such as GLP-1), are able to preserve beta-cells. However, these inhibitors also act by reducing hepatic glucose production, while stimulating insulin secretion and decreasing glucagon secretion [DeFRONZO, 2009].

1.4 Obesity and T2DM

Worldwide, obesity prevalence more than doubled between 1980 and 2008. In 2008, 10% of men and 14% of women in the world were obese (BMI ≥ 30.0 Kg/m^2) and 35% of adults older than 20 years were overweight (BMI ≥ 25.0 and < 30 Kg/m^2).

Recent studies [ECKEL, 2011] have found associations between T2DM and obesity involving pro-inflammatory cytokines (tumor necrosis factor, interleukin-6), insulin resistance, changes in fatty acid metabolism, and cellular processes such as mitochondrial dysfunction and endoplasmic reticulum stress.

These interactions are complex and their importance has not been clearly defined. Nevertheless, there is no doubt that obesity plays a major role in the pathogenesis of diabetes. This relationship can be observed when analyzing data from epidemiological studies.

In the U.S. population, between the ages of 20 and 74 years there was a significant increase in the prevalence of diabetes between 1976–1980 and 1999–2000 (3.3% to 5.8%). However, these trends varied according to the BMI level. In individuals with BMI ≥35 kg/m^2, diabetes increased considerably (from 4.9% in 1960, to 8.6% during 1976–1980, to 15.1% in 1999–2000). [GREGG, 2004]

Therefore, the proportion of diagnosed diabetes cases increased from 41 to 83% among individuals with BMI ≥35 kg/m^2, while changes in diabetes prevalence in subjects with lower BMI's were modest, leading to imperceptible changes in the total number of diagnosed cases. [GREGG, 2004]

There is evidence that the common link between obesity and diabetes is the proinflammatory state usually seen in patients with metabolic syndrome [MARINOU, 2012].

The metabolic syndrome is defined as a conjunction of metabolic risk factors, as follows [HUMPHREY, 1998; SCOTT, 2004]

- atherogenic dyslipidemia with serum elevation of triglycerides, apolipoprotein B and small low-density lipoprotein (LDL) associated with low high-density lipoprotein (HDL) cholesterol;
- elevated blood pressure;
- elevated glycemia and insulin resistance;

- proinflammatory state;
- prothrombotic state.

The National Cholesterol Education Program Adult Treatment Panel III report proposed the following criteria for diagnosis of metabolic syndrome (3 of the 5 following criteria must be met for its diagnosis) [NCEP, 2002]
- increased waist circumference (≥ 102 cm in men and ≥ 88 cm in women);
- elevated tryglicerides (≥ 150 mg/dL);
- reduced HDL cholesterol (< 40 mg/dL in men and < 50 mg/dL in women);
- elevated blood pressure (≥ 130x85 mmHg or on treatment for hypertension);
- elevated glucose (≥ 100 mg/dL).

1.5 Surgical Treatment of T2DM

Gastric bypass and other types of bariatric surgery have shown to be effective in the treatment of T2DM, reducing the mortality rate in the long term when compared with optimal clinical treatment [BUCHWALD, 2009; SJOSTROM, 2004, 2007] .The term "bariatric" is gradually being replaced by "metabolic", since the surgery previously recommended for the treatment of morbidly obese individuals (defined by BMI greater than 40 kg/m^2 or greater than 35 kg/m^2 associated with comorbidities that are difficult to control) has demonstrated excellent results in terms of diabetes remission, even in patients with BMI lower than 35 kg/m2. Thus, the use of BMI as the single criterion for indication for surgical treatment has been questioned by several medical associations, especially for diabetic patients with BMI < 35 kg/m2 and whose disease is difficult to control using pharmacological treatment.

Therefore, a "metabolic" surgery is currently defined as any surgical procedure in which there is any anatomical alteration in the gastrointestinal tract by means of a diversion of food passage, resulting in improved metabolic control in patients with T2DM [SCHULMAN, 2009]. Such result does not depend on weight loss and, in some cases, it can be observed some days or weeks after the surgical procedure, long before considerable weight loss. A growing number of these surgeries are being performed every year worldwide, and they are expected to be part of the algorithms for diabetes therapy, combined with changes in lifestyle and drug therapy. [DIXON, 2011]

Several studies have shown that the best results regarding T2DM remission are obtained after procedures that include proximal bowel diversion, performed in the Roux-en-Y gastric bypass or biliopancreatic diversion. In a 14-year follow-up, T2DM remission was found in 83% of the patients who underwent gastric bypass [PORIES, 1995]. Similar data were found in 240 patients undergoing the same type of surgery, with 83% remission in a mean follow-up of 10 years [BUCHWALD, 2004]. Biliopancreatic diversion, in turn, offers superior results regarding T2DM remission, found in 97% of patients with a mean follow-up of 10 years. However, there is a greater number of long-term complications, especially nutritional complications (15 to 20% of malnutrition in the long term) [BUCHWALD, 2004; PORIES, 2008]. In this context, Roux-en-Y gastric bypass carries more advantages because it is a more standardized surgery, with longer follow-up studies, showing a low

mortality rate (0.30%), with few major complications in the long term (0.7%) [BUCHWALD 2004]. Restrictive procedures, such as adjustable gastric band, have lower rates of T2DM remission (45%). Such results are dependent on caloric restriction and weight loss and are not maintained in the long term, when is weight recovery is common. [DIXON, 2008]

Studies have shown that the mechanisms responsible for metabolic improvement after a Roux-en-Y gastric bypass include weight loss, decreased insulin resistance, increased insulin production, proximal bowel diversion, increased incretins levels, and bile flow alteration [LeROUX, 2006; BORG, 2006; LeROUX, 2007; THALER, 2009; POURNARAS, 2010]. In relation to renal function, other studies also found increased urine production and sodium excretion after gastric bypass in rats [BUETER, 2011], as well as improvement of urinary and systemic inflammatory markers in patients [BUETER, 2010]. A retrospective study involving 83 T2DM patients with previous nephropathy and/or retinopathy showed that, after gastric bypass surgery, nephropathy and retinopathy remission were very common, with an 85.2% reduction in the albumin/creatinine ratio and improvement in retinal images in 19% of cases (manuscript in preparation).

In recent decades, T2DM therapy has not been exclusively aimed at controlling glycemic levels, but is instead focused on preventing macrovascular and microvascular complications (ISMAIL-BEIGI, 2011].

Every new T2DM treatment must be safe and effective, not only to correct hyperglycemia, but also to prevent or mitigate the complications of this chronic disease. Only one prospective study showed that biliopancreatic diversion can reverse diabetic nephropathy [IACONELLI, 2011]. This surgery is not commonly performed worldwide because of the nutritional complications in the long term. The only randomized controlled trial conducted in patients with T2DM undergoing bariatric surgery focused only on glycemic control. Furthermore, in this study, patients underwent placement of adjustable gastric band [DIXON, 2008], showing improvement in glycemic control directly related to weight loss with inferior results in comparison with other surgical techniques.

Roux-en-Y gastric bypass is considered the gold standard for bariatric surgery, and it is the type of surgery most often performed in the world to control obesity [SCHAUER, 2003]. It consists of gastric volume reduction and exclusion of duodenum and proximal jejunum from food transit. As it has been found in several studies [PORIES, 1995; RUBINO, 2002; RUBINO, 2004], this type of operation promotes reduction of glucose levels in diabetic patients a few days after the procedure, even before there is an effective weight loss. Based on these findings, it was possible to suggest that the T2DM control mechanism achieved by this procedure occurs mainly as a result from the alteration in the normal gastrointestinal transit, instead of resulting exclusively from weight loss. Recently this group of researchers [Cohen, 2012] published the long-term results of surgical treatment for T2DM in patients with BMI between 30-35 kg/m^2. After 6 years of follow-up, has been shown that 88% of 66 patients operated got on remission, i.e. without antidiabetic and HbA1c less than 6.5% and control of dyslipidemia and hypertension.

Some hypotheses have been proposed to explain the effect of this surgery on T2DM. The most accepted hypothesis is related to the influence of the duodenum and proximal jejunum (proximal bowel mechanism) as key factors in the control of insulin resistance in

T2DM patients. Rubino (2004), in an experimental study with a model of duodenal exclusion in nonobese diabetic Goto Kakizaki rats, demonstrated impressive improvement in glycemic levels of these animals compared with the control group. In another study (RUBINO 2006), the same author concluded that duodenal-jejunal exclusion was more important than ileal stimulation (distal bowel mechanism) for the normalization of glucose levels in these animals. Rubino suggested that a hormone secreted by the duodenum has an "anti-incretinic" effect and with the exclusion of this intestinal segment, this blockade would disappear and the opposite effect would dominate, i.e., reduced insulin resistance and increased insulin secretion.

Another hypothesis postulates that the rapid arrival of nutrients in the ileum (distal intestine mechanism) strengthens the effect of GLP-1, which is an incretin hormone secreted by ileal L cells in response to the arrival of undigested food. This hormone would stimulate insulin secretion and have an antiapoptotic effect on pancreatic beta-cells, as mentioned above, leading to glycemic control and preservation of pancreatic endocrine cells [MOO & RUBINO, 2008; LEE, 2008; RUBINO, 2006; RUBINO, 2002]. Roux-en-Y gastric bypass produces the two mechanisms explained above.

These studies also showed that the hypothesis of weight loss especially resulting from lower intake of carbohydrates and lipids has been demystified, since D-xylose and fecal fat tests showed no significant difference when compared with controls [RUBINO, 2006].

However, it seems that there is a change in the metabolism of free fatty acids, which, in high concentrations, lead to insulin resistance. In addition, after the surgical procedure, reduction of free fatty acids may have an influence on the improvement of insulin resistance [SNOW, 1993; BOURDAGES, 1994].

Gastrointestinal anatomy rearrangement may also be related to the control of T2DM based on the effect of other hormones. Some of these hormones are: peptide YY (PYY) and GIP, which are secreted in the proximal and distal bowel and are also related to the beta-pancreatic functioning and satiety; as well as ghrelin, which acts as an orexigenic signal and it is secreted in gastric fundus and duodenum. Besides that, ghrelin has direct and counter regulatory diabetogenic effects [DE PAULA, 2006; COHEN, 2006 e 2012].

These assumptions were also tested in studies involving humans with BMI < 35 Kg/m² who underwent duodenal exclusion [COHEN, 2007] and ileal interposition [DE PAULA, 2006]. The initial results from these studies seem promising and suggest a new possibility of T2DM control in patients with overweight and grade 1 obesity. What is really interesting regarding these satisfactory results is that there is evidence of improvement in the beta-cell function of diabetic patients who underwent surgery when compared with a group of patients with normal glucose tolerance, and that glycemic control was mainly caused by a direct antidiabetic unrelated to the patients' weight change [KLEIN & COHEN, 2012].

2. RATIONALE

2.1. Surgery in patients with low BMI

Excellent results observed after gastric bypass in patients with diabetes and BMI> 35 kg/m^2 along with evidence of weight-independent mechanisms antidiabetic led to considering surgery to less obese diabetics. Clinical practice of bariatric surgery, however, is based on a 1991 NIH consensus, which limits the use of bariatric surgery in patients with BMI ≥ 35 kg / m2 with comorbidities, such as diabetes.

Patients with BMI between 30 and 35 kg/m2 (obesity grade I) are most diabetic patients. Millions of these suffer from diabetes uncontrolled despite lifestyle changes and pharmacotherapy; and does not enter the NIH criteria for bariatric surgery. Therefore, we propose to evaluate the effect and safety of this surgery for type 2 diabetic patients with obesity grade I.

2.2. Surgery x Clinical treatment

The medical community is confronted with many different studies using various methodologies to investigate the best pharmacological treatment for T2DM. The treatment algorithm offers several different options according to the stage of the disease (which is different in each study). In addition, new drugs are being developed over the years, but are not always a guarantee of effective T2DM control [MENDES, 2010]. Furthermore, these drugs do not prevent the development of this disease, consequently increasing the risks of microvascular and macrovascular complications.

Conversely, there is considerable evidence that surgery can be an adequate tool to promote T2DM remission in patients who are unresponsive to clinical treatment. Gastric bypass surgery is one of the most popular bariatric surgeries in the world, but its effects on microvascular and macrovascular complications of T2DM have not been established. Specialists suggest that the rapid and uncontrollable decrease in blood glucose adds to the concern that the surgery may paradoxically cause exacerbation of microvascular complications [LEOW, 2005], whereas gradual improvement in blood glucose before gastric bypass surgery may prevent this paradoxical worsening, leading to an interruption of this process, or even retinopathy, nephropathy, and neuropathy remission.

However, there are no studies comparing the results of these two types of treatment (clinical vs. surgical) in a similar population and assessing the development of microvascular complications of T2DM. Therefore, in order to clarify such doubts, it is necessary and extremely desirable to conduct a randomized controlled trial comparing gastric bypass with the best and most modern clinical treatment. Its findings could have a direct impact on hundreds of millions of diabetics by allowing the inclusion of surgical treatment as a safe and feasible therapeutic option for a significant portion of these patients.

3. STUDY AIM

The aim of this prospective, open, randomized study is to evaluate the effects of Roux-en-Y gastric bypass in the control of diabetic nephropathy in diabetic patients with BMI between 30 and 35 kg/m^2.

4. ENDPOINTS

4.1. PRIMARY ENDPOINT

The primary endpoint will be the proportion of patients that present normalization of the albumin/creatinine ratio in isolated urine samples (normal value considered as an albumin/creatinine ratio of less than 30 µg/mg).

4.2. SECONDARY ENDPOINTS

1. Retinopathy reversal
2. Development or worsening of peripheral neuropathy
3. Discontinuation of pharmacological therapy for T2DM
4. Glycemic control (fasting glucose level < 100 and HbA1c < 6.5%)
5. Normalization of blood pressure (systolic blood pressure < 130 mmHg and diastolic blood pressure < 80 mmHg) (ADA, 2012]
6. Normalization of lipids (LDL < 100 mg/dL and < 70 mg/dL in patients with cardiovascular disease; HDL > 50 mg/dL, triglycerides < 150 mg/dL) (ADA, 2012]
7. Improvement on Quality of life (SF-36)
8. Improvement of voiding dysfunction

5. STUDY DESIGN AND STATISTICAL ANALYSIS

5.1. STUDY DESIGN

This is a prospective, open, randomized study involving 100 patients with microvascular complications of T2DM and obesity, who will undergo gastric bypass (Roux-en-Y gastric bypass ARM A) or receive best medical treatment (ARM B, control arm).

Fifty obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited as surgeon of excellence by the

SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

On randomization, months 6,12, 28,40 and 52 visits patients allocated in the surgical arm will be submitted to elastography ARFI (Acoustic Radiation Force Impulse). The method quantifies the degree of fibrosis with high accuracy, with the advantages of being coupled to a conventional ultrasound equipment (Siemens S2000), allowing the visualization of the liver and determining the liver segment to be analyzed.

The literature, including the first national study pilot shows high accuracy in quantifying the degree of fibrosis compared to liver biopsy in hepatopathy, nonalcoholic steatohepattis, steatohepatitis and other numerous liver diseases. [Junior, 2012; Friedich-Rust, 2012; Palmeri, 2011; Boursier, 2010]

The method has been used routinely in many countries and has been introduced in our country, in order to reduce the number of liver biopsies. The elastography tests will be carried out in Schmillevitch Diagnostics Center - São Paulo, by a single operator.

Compared to the diagnostic method mentioned above, during the surgery will be performed Biopsy Liver in order to quantify the degree of hepatic steatosis.

In the randomization and months 6,12,24,36,48 and 60 visits will be collected 3 to 5 ml of urine, in both study arms. In these samples, DNA sequencing will be searched for correlation of urinary metabolites with the success of treatments in the control of secondary microvascular disease to diabetes. The samples will be analyzed at the Santa Casa de Misericordia - São Paulo and will be stored until the end of the study. This collection also will be included in the Informed Consent Form that will be signed by the research participant.

Regarding medication to be used, if there is no contraindication, metformin will be maintained in the postoperative period while fasting glycemia is above 100 mg/dL. Anti-antihypertensive drugs and medications for dyslipidemia will be maintained in the postoperative period, unless there are any contraindications. A supplement of micronutrients (vitamins and mineral salts) will be prescribed to all patients who undergo surgery. Patients allocated to the control group will receive the same supplementation if necessary.

The control group will include fifty patients with microvascular complications of T2DM who will receive the best clinical treatment available, which will consist of metformin, glitazones, incretin therapy (DPP4 inhibitor and GLP-1 analogs) and insulin, if necessary [DeFRONZO, 2009]. In addition, other comorbidities, such as hypertension and dyslipidemia, will be compensated according to ADA recommendations [ADA, 2012].

5.2. SURGICAL PROPOSAL

1) Pneumoperitoneum closed with Veress needle
2) Identification of Treitz angle
3) Measurement of biliary loop (100 cm)
4) Bowel transection with linear stapler (white load)
5) Measurement of small intestine (150 cm)
6) Laterolateral Entero-anastomoses (white load)

7) Construction of gastric pouch distant about 3 cm from the esophageal-gastric junction with stomach section in the small curvature.
8) Linear cutting anastomosis (gastrojejunostomy) from about 1 to 1.2 cm
9) Anastomosis integrity evaluation by methylene blue test and/or perioperative air.

Expected surgical time: 60 minutes

5.3. INCLUSION CRITERIA

- Male and female adult patients with microalbuminuria (more than 30 mg and less than 300 mg or more of urinary albumin per 24hours), with or without other microvascular complications of T2DM, receiving pharmacological treatment for the disease, which may or may not include the use of insulin;
- Age between 18-65 years;
- BMI between 30 and 35 Kg/m^2;
- 15-year or less after T2DM diagnosis;
- Negative anti-GAD;
- Fasting C-peptide higher than 1 ng/ml, increasing in the postprandial period (two hours after mixed meal, ENSURE plus approximately 500 Kcal).

5.4. EXCLUSION CRITERIA

- Patient's refusal to participate;
- Autoimmune DM;
- Previous abdominal surgeries that may make surgery more difficult, increasing the surgical risk;
- Previous malabsorptive and restrictive surgeries;
- Pregnant women and nursing mothers;
- Recent history of neoplasia (< 5 years), except for non-melanoma skin neoplasms
- History of liver disease – liver cirrhosis –, active chronic hepatitis, active hepatitis B and hepatitis C;
- Malabsorptive syndromes and inflammatory bowel disease;
- Cardiovascular event (acute myocardial infarction, acute coronary syndrome, angioplasty, or bypass in the last 6 months);
- Angina;
- Pulmonary embolism or severe thrombophlebitis in the last 2 years;
- Positive HIV serum testing;
- Psychiatric disorders, including dementia, active psychosis, severe depression, history of suicide attempts, use of illicit drugs, and excessive alcohol consumption in the last 12 months;
- Uncontrolled coagulopathy;
- Patients with severe retinopathy, nephropathy, and neuropathy (defined as high risk/advanced proliferative retinopathy or amaurosis; stage 5 of chronic kidney disease

defined by glomerular filtration rate, patients who need dialysis or renal transplantation; stage 3 of peripheral neuropathy);
- Patients who participated in other clinical trials in the past 30 days.

5.5. RECRUITMENT & FOLLOW-UP

The recruitment period will last for 2 years (24 months) from the beginning of the study (initiation visit), and follow-up will proceed for 60 months after participant inclusion in the study.

5.6. STATISTICAL ANALYSIS AND SAMPLE SIZE CALCULATION

5.6.1. STATISTICAL ANALYSIS

All analyzes will be performed according to the intention-to-treat principle. Missing data will be entered using previous approaches if appropriate [PEDUZZI 2002].

Data will be expressed as mean ± standard deviation (SD), median (interquartile range), or absolute number (or percentage), when appropriate.

For continuous data, independent groups will be compared by an unpaired t test for approximately normally distributed variables (with Welch's correction when deemed necessary), or by means of the Mann-Whitney U-test for variables with skewed distribution. Within-group (pre- vs. post-) differences will be investigated by the t test for paired samples or by Wilcoxon's matched-pairs signed-ranks test. Fisher's exact test will be employed to test differences in count data. In addition, multiple logistic regression models using the backward stepwise selection procedure (cutoff ≤ 0.10) will be fit to ascertain potential predictors for binary variables. A similar approach will be applied for continuous dependent variables using multiple linear regression models. All data analyses will be performed using the Stata package (version 11.0, Stata Corp., College Station, TX, USA). Statistical significance level will be set at 5% (two-tailed).

5.6.2. SAMPLE SIZE CALCULATION

Previous estimates for the proportion of participants who are likely to achieve the primary endpoint (that is, a normal albumin/creatinine ratio) in the group allocated to clinical treatment (Pc) and in the group of surgical intervention (Ps) were obtained in the medical literature (BOUSSAGEON, 2011) and unpublished data of the group.

We determined the sample size required to detect an association with a 5% level of significance (α) assuming a randomized controlled trial with two independent groups.

This approach assumes that the null hypothesis (that $\Delta = 0$, where $\Delta = Ps - Pc$) will be accepted/rejected on the basis of a 2-sample test for equality of proportions with continuity correction data.

The statistical power was first calculated analytically in accordance with the method described by ZAR (1999). Analytically derived estimates of the power of the study were corroborated with an empirical approach, based on the Monte Carlo simulation. With this

purpose, individual studies were simulated according to the methodology proposed by SUTTON et al. (2007) and data were analyzed using Fisher's exact test when the asymptotic assumptions were not valid (for example, counts lower than 5 in the 2x2 table). In the remaining cases, we used the Z test, where Z =θ/IF(θ) and θ and IF(θ) are, respectively, the estimated logarithm of odds ratio and its asymptotic standard error. The empirical power was calculated as the proportion of 10,000 simulations that produced a two-tailed p value < 0.05. All analyzes were performed using the statistical package Stata 11.0 (Stata Corporation, College Station, TX, USA).

Thus, considering the data in the literature and unpublished data of the group, as well as a rate of loss to follow-up of 15% for both groups and a two-tailed type I error (α) of 5%, the final scenario is:

Statistical power of 90%

The final scenario underlying the increase of the segment and following conjecture a loss of 38%, assuming the following probabilities:

Pc = 10% (probability of achieving the primary endpoint in the clinical group)

Pc = 50% (probability of achieving the primary endpoint in the surgical group)

As the best option to answer the question of this protocol, and assuming a conservative profile as a guarantee to obtain reliable results, we chose the conditions Ps and 50% PC 10%, with a statistical power of 90% (final stage). These parameters show an optimal number of research subjects in each arm of 50 patients, reaching a total of 100 patients in the study.

5.6.3. RANDOMIZATION AND ALLOCATION OF PATIENTS

Eligible patients will be randomly assigned with concealed allocation to either bariatric surgery or diet/lifestyle intervention. A biostatistician not otherwise associated with the study will be responsible for generating the computerized randomization schedule. An independent research assistant will manage and schedule the communication between participants and physicians.

Randomization process will be accomplished by the computer-generated permuted using block approach. Specifically, random blocks with predetermined sizes of at least two patients, at the latest four patients will be generated.

Concealed allocation will be ensured through the use of random IDs (group ID). These IDs will be used to name the patients, so that does not contain personal information. So that, through a randomization, clinical research coordinator will send to the statistical gender identification number (male/female). The statistical returns only referencing the group that patients were allocated (Group 1: surgery, Group 0: clinical).

5.6.4. STUDY FLOW CHART

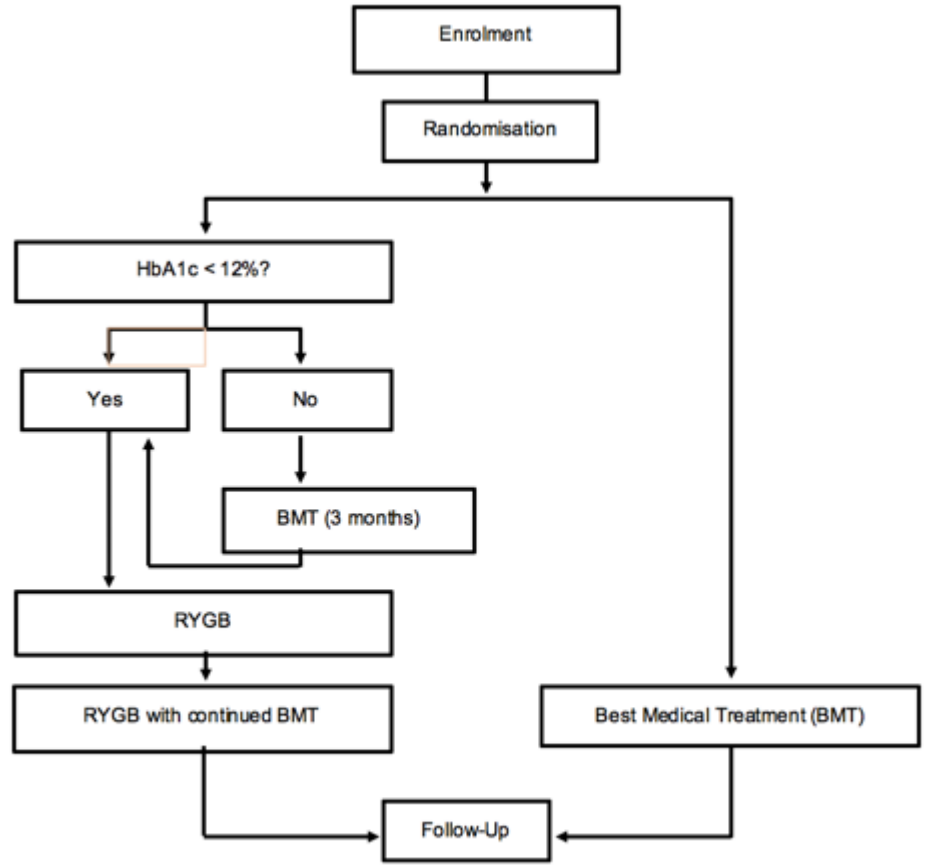


Figure 1. Simplified MOMS algorithm

5.6.5. CRITERIA FOR PATIENTS' WITHDRAWAL FROM THE STUDY

If, at any time, significant problems with the surgery are suspected, a re-operation may be performed. Should participants require withdrawal for any reason, follow-up phone calls will continue as scheduled. If a patient cannot be reached, then friends or relatives of the patient will be contacted,

Patients may withdraw of the study if:

- voluntary decision to withdraw;
- study is terminated.

6. STUDY PROCEDURES

Patients will undergo procedures according to the time intervals described in the Procedures Flow Chart (item 7.5).

6.1. SELECTION/SCREENING

The screening period will begin after the subjects voluntarily sign the informed consent form. Those subjects who provide consent will undergo the following screening procedures to determine their eligibility for the study:

- Medical anamnesis, which will include collection of information for the assessment of the subjects' clinical history, collection of demographic data (date of birth, sex, race, etc);
- Evaluation of inclusion and exclusion criteria;
- Medical examination of the subjects' overall clinical status;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, eyes, ears, nose, throat, heart, lungs, breasts, abdomen, external genitalia, limbs, muscles, and neurological system;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start date;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey.
- Quality of life assessment using the SF-36;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Baseline Visit;
- Laboratory tests as described in the Flowchart of the Study and in Assessments and Tests;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.
- Chest x-rays, thoracic, anteroposterior and lateral projections;
- Full abdominal ultrasound, under fasting and bladder repletion;
- Standard digital 12-lead electrocardiogram, including heart rate, rhythm, and RR, PR, QRS and QT intervals;
- Color Doppler echocardiogram. If significant changes are found, the physician in charge might decide to refer the subject to dypiramidol myocardial perfusion scintigraphy to better establish the patient's clinical picture;

- Color retinography, angiofluoresceinography and refraction test to evaluate the presence of diabetic retinopathy;
- Vital signs checking, including weight, height, heart rate, blood pressure, axillary temperature, and respiratory frequency.

6.2. BASELINE VISIT

-Evaluation of all laboratory tests and imaging results;
- If the subject is found elegible for the study (meet all inclusion criteria and no exclusion criteria), the subject will be randomized for the study. The randomization procedure to be performed is described in Section 6.5.3;
- Subjects randomly assigned to the intervention group (surgery) who present HbA1c ≥ 12,0% will be clinically treated during 3 (three) months and will be reevaluated in a new baseline visit. If during this new baseline the subject still presents HbA1c ≥ 12,0%, he will be excluded from the study.
- Upper digestive endoscopy only for patients randomized to intervention group, including H. pylori infection investigation and biopsy only if necessary;
- Elastography;
- At reevaluation, subjects should redo the following exams, procedures or assessments: medical evaluation, physical exam, nutritional assessment, serum pregnancy test if applicable, blood count, AST/ALT, sodium, potassium, urea, creatinine, coagulation, lipid profile, fasting glucose, HbA1c, urinalysis, urine type I, microalbuminuria, urinary creatinine, electrocardiogram, chest X-ray, vital signs measurement, weight and height.
- Dispensing of drugs and materials needed for the subject until next visit;
- Provide information about surgical procedure for subjects randomized to the intervention group (surgery procedure);
- Guide patients to check blood glucose using a blood glucose meter that will be provided by the Health Research Unit. Blood glucose should be checked daily in the morning on an empty stomach and at night two hours after dinner. Patients who are considered glycemic controlled (fasting ≤ 150mg/dL and postprandial ≤180mg/dL) should measure blood glucose only 3 times a week;
- Provide information about surgical procedure and drug treatment for subjects randomized to the intervention group (surgery procedure);
- Provide information about drug treatment for subjects randomized to the control group (clinical treatment);
- Urine sample collection for DNA sequencing;
- Collect voiding diary to control urinary habit, provided in the Screening visit.

6.3. SURGERY

- Up to 3-day hospitalization for surgical procedure. Those subjects randomized to the surgical arm of the study will be admitted to individual rooms and will receive full care during the period of hospitalization;
- Gastric bypass as described in the item 5.5 (Surgery plan);

- Hepatic biopsy during surgery: the extracted fragment will be forwarded to pathological analysis, as described in the item 6.1;
- Laboratorial exam collection: RCP;
- Medical examination of the subjects' overall clinical status;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, heart, lungs, abdomen and neurological;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Vital signs checking, including weight, height, heart rate, blood pressure, axillary temperature, and respiratory frequency.

6.4. CLINICAL FOLLOW-UP

6.4.1. First to third postoperative day (surgical arm patients)

-Medical examination of the subjects' overall clinical status;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, heart, lungs, abdomen and neurological;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit. All patients will use Ranitidine hydrochloride (150mg/day).

6.4.2. Week 1 Visit (intervention arm patients)

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;

- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey.
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose;
- Dispensing of materials and drugs needed until the next visit.

6.4.3. Visits - Week 4, Month 3, Month 9, Month 15 and Month 21

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, heart, lungs, abdomen and neurological;
- Assessment and review of any adverse event occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Monh 6 (only on Month 3);
- Dispensing of materials and drugs needed until the next visit. All patients in the surgical arm will use multivitamin supplements daily until the end of the study.

6.4.4. Visits - Month 6 and Month 18

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, heart, lungs, abdomen and neurological system;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;

- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Color retinography, angiofluoresceinography and refraction test to evaluate the presence of diabetic retinopathy;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF (only on Month 6);
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 12 Visit (only on Month 6);
- Urine sample collection for DNA sequencing;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose;
- Dispensing of materials and drugs needed until the next visit.

6.4.5. Month 12 and Month 24 Visits

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;
- Color retinography, angiofluoresceinography and refraction test to evaluate the presence of diabetic retinopathy;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;

- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 24 and Month 36 Visit, consecutively;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

6.4.6. Month 28 Visit

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Assessment of capillary glucose;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

6.4.7. Month 32, Month 44 and Month 56 Visits

- Medical examination of the subjects' overall clinical status;

- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Assessment of capillary glucose;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

6.4.8. Month 36 Visit

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 48;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;

- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

6.4.9. Month 40 and Month 52 Visits

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

6.4.10. Month 48 and Month 60 Visits

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;

- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 60;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit (only on Month 48).

7. Procedures Flowchart

	Year 1										Year 2				Year 3			Year 4			Year 5		
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24	M28	M32	M36	M40	M44	M48	M52	M56	M60
Informed consent	X																						
Anamnesis	X																						
Inclusion/exclusion criteria	X	X																					
Medical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X																					
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X							X		X		X		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nerve conduction studies	X							X		X		X		X	X	X	X	X	X	X	X	X	X
Nutritional assessment	X				X	X	X	X	X	X	X	X	X	X	X		X	X		X	X		X
SF-36 administration	X									X				X			X			X			X
I-PSS assessment	X									X				X			X			X			X
OAB-qSF assessment	X									X				X			X			X			X
Dispensation - voiding diary	X						X	X		X				X			X			X			X
Serum pregnancy test	X																						
Elastrography*		X						X		X					X			X			X		
Complete blood count	X									X		X		X			X			X			X
AST/ALT	X									X				X			X			X			X
Amylase	X							X***				X***			X			X			X		
Sodium and potassium	X																						
Urea and creatinine	X						X	X		X		X		X	X	X	X	X	X	X	X	X	X
Coagulogram (PT and APTT)	X																						
Lipid profile	X						X	X		X		X		X	X		X	X		X	X		X
Fasting glycemia				X	X	X	X		X		X		X		X	X	X	X	X	X	X	X	X
HbA1c	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Microalbuminuria alone	X							X		X		X		X	X		X	X		X	X		X
Urinary creatinine	X							X		X		X		X	X		X	X		X	X		X
Iron and ferritin	X							X		X		X		X			X			X			X
HOMA	X							X		X		X		X			X			X			X

	Year 1										Year 2				Year 3			Year 4			Year 5		
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24	M28	M32	M36	M40	M44	M48	M52	M56	M60
Meal tolerance test	X							X		X		X		X	X		X	X		X	X		X
Ionazable calcium	X							X		X		X		X			X			X			X
PTH	X							X		X		X		X			X			X			X
Vit D (25 OH)	X							X		X		X		X			X			X			X
Vit A	X							X				X		X			X			X			X
Folic acid	X							X						X			X			X			X
Vit B1	X							X				X		X			X			X			X
Vit B12	X							X				X		X			X			X			X
Anti-GAD	X																						
Urine I	X									X				X			X			X			X
RCP			X*				X*	X*		X*		X*		X*	X*		X*	X*		X*	X*		X*
Urine culture																							
Echocardiogram	X																						X
Urine for DNA sample		X						X		X				X			X			X			X
PSA	X																						
Anti-HIV	X																						
Chest x-ray	X																						
Abdominal ultrasound	X																						
Electrocardiography	X																						
Upper digestive endoscopy		X*																					
Color retinography		X						X		X		X		X									
Angiofluoresceinography		X						X		X		X		X									
Refraction test		X						X		X		X		X									
Vital signs	X		X	X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X			X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																						
Drug dispensing	X		X	X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Glucose assessment					X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gastric bypass			X																				
Hospitalization			X																				

* Applicable for patients randomized to the intervention group (surgical procedure).
** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days prior to randomization visit (baseline), for patients in the clinical arm and from the surgery for patients in the surgical arm.
*** Applicable for patients randomized to the clinical group

8. ASSESSMENTS AND TESTS

Laboratory and imaging tests will be performed on the premises of Oswaldo Cruz Hospital and managed by Fleury Medicine and Health group, currently the largest center of diagnostic medicine in Brazil. The institution has the necessary national and international certifications, such as ISO 9001, ISO 14001, American College of Radiology, College of American Pathologists, Proficiency in Laboratory Tests of the Brazilian Society of Clinical Pathology/Laboratory Medicine, and the NGSP level I certificate of traceability to the DCCT reference method for assessment of metabolic parameters.

Only retinography and elastography will be performed in outpatient clinics. These were previously audited in relation to diagnostic method and specificity study.

8.1. RENAL FUNCTION

It will be measured before and 6, 12, 18, 24, 28, 36, 40, 48, 52 and 60 months after inclusion in the trial. The glomerular filtration rate will be estimated using the Cockcroft-Gault equation and the Modification of Diet in Renal Disease (MDRD) equation.[34] Albuminuria will be measured and the albumin/creatinine ratio will be calculated in isolated urine samples collected in the morning. Patients will be instructed to avoid physical activity the day before the collection. Urine I will be collected at time points 0, 1 year and 2 years after inclusion in the trial, and if necessary (presence of leukocyturia), urine culture will be performed.

8.2. PERIPHERAL NERVOUS SYSTEM FUNCTION

The sensory and motor function of the peripheral nervous system will be assessed by nerve conduction studies (10-g monofilament and 128-Hz tuning fork)[33] before and 6, 12, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56 and 60 months after inclusion in the trial.

8.3. RETINAL ASSESSMENT

Retinopathy will be assessed and compared by colored retinography, angiofluoresceinography and refraction test performed before and 6, 12, 18, and 24 months after inclusion in the trial. If indicated by the ophthalmologist, fluorescein angiography will be performed. This test can detect early changes in diabetic retinopathy.[35]

8.4. USE OF MEDICATIONS

All medications used will be clearly recorded, including trade and generic name, dose, and drug administration regimen. These data will be obtained by the researchers at each patient visit during follow-up. Treatment adherence will be determined based on the number of tablets and units of insulin given to the patient. At each visit, the patient will have to show to the researchers whether and how many tablets/units are left.

A scale of medication use will be calculated based on the number of medications and their dosage. For each oral medication, a numerical score will be calculated based on the ratio between daily and maximum recommended dose [UPTODATE, 2011]. In cases of insulin users, a numerical score will be calculated based on the ratio between daily insulin

dose and the standard dose of 1 U/kg/day. The final score will be obtained for each patient by the sum of the scores of each medication.

8.5. ASSESSMENT OF GLYCEMIC CONTROL

All patients will undergo measurement of fasting glycemia and HbA1c before and 3, 6, 9, 12, 15, 18, 21, 24, 28, 32, 36, 40, 44, 48, 52, 56 and 60 months after inclusion in the trial. Fasting glycemia will also be measured one day, one week and one month after surgery. Patients will receive guidance regarding capillary glycemia performed 3 times a day for patients using insulin and twice a week for the other patients.

8.6. ASSESSMENT OF BLOOD PRESSURE

Blood pressure will be measured at each patient visit using a calibrated device, adjusted for arm circumference for each patient. The patient will be seated for at least 5 minutes before each measurement. An average of two measurements will be recorded.

8.7. ASSESSMENT OF LIPID PROFILE

Total cholesterol, LDL, HDL, and triglycerides will be measured in blood samples collected in the morning after a 10-hour overnight fast before and 3, 6, 9, 12, 18, 24, 28, 32, 36, 40, 44, 48, 52, 56 and 60 months after inclusion in the trial.

8.8. ASSESSMENT OF QUALITY OF LIFE

Quality of life will be assessed by the SF-36 (Medical Outcomes Study 36 – Item short form health survey), translated and validated in Brazilian Portuguese [IQOLA SF-36, 1996], before and 12, 24, 36, 48 and 60 months after inclusion in the trial.

8.9. ASSESSMENT OF TREATMENT COMPLICATIONS

All complications and adverse events will be clearly recorded and classified according to severity at each patient visit [CTCAE, 2009].

8.10. ESTIMATE OF MEDICATIONS AND CAPILLARY GLYCEMIA REAGENT STRIPS USED

Product	Surgical group (n=50)	Clinical group (n= 50)
Metformin 1g	4200 cp/month	4200 cp/month
Actos 30 mg	420 cp/month	630 cp/month
Linagliptin 5 mg	420 cp/month	630 cp/month
Liraglutide 6.0 mg/mL	5 pens/month	100 pens/month
Lantus	10500 IU/month	21000 IU/month
Apidra	1300 IU/month	5000 IU/month
Centrum	2100 cp/month	
Reagent strips	4000/month	4000/month

8.11. Evaluation of voiding dysfunction:

In addition to the microvascular complications such as nephropathy, retinopathy and neuropathy, diabetic bladder dysfunction is described also known as diabetic cistopatia consisting of changes dependent on the retention time and bladder emptying, is the most important complication of lower urinary tract due to T2DM and correlated with oxidative stress caused by this [KEPABSCI, 2007; DANESHGARI 2009]. Still, there is evidence that weight loss in obese induced by bariatric surgery, promotes significant improvement in symptoms of lower urinary tract, such as urinary incontinence, stress or urgency, both in women as in men. Thus, we propose a voiding evaluation of these patients through two questionnaires and record in voiding diary for control of urinary habits.

Voiding dysfunction evaluation as a complication of T2DM in mild obese patients will be assessed by applying two questionnaires and reporting in a voiding diary to control urinary habit, as described below:

- Application of questionnaires IPSS (International Prostate Sympton Score) and OAB-q (Overactive Bladder Questionnaire short form), both with validation for the Portuguese language and self-applicable. Although originally intended for evaluation only of prostatic symptoms in men, the IPSS has been applied to women, as the clinical signs of prostate problems (symptoms of lower urinary tract) may also occur due to other common comorbidities to both sexes [OKAMURA, 2009; HSIAO, 2013; CHOI, 2014].

Questionnaires will be applied at baseline and after 6, 12, 24, 36, 48 and 60 months of intervention. Dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days for the next visit (month 12, 24, 36, 48 and 60)

The comparison will be made between the initial time and the subsequent.

8.12. SAFETY ASSESSMENT

Safety will be assessed by clinical evaluations, laboratory tests, vital signs, and adverse events.

Assessment of adverse events will begin upon signature of the informed consent form by the patient and will end 30 days after month-24 patient visit. If the patient withdraws from the trial before month-24 visit, adverse events will be collected for 30 days after the withdrawal date.

8.12.1. Adverse Event

An adverse event is any unexpected medical occurrence in a research subject of a clinical trial. It does not necessarily imply any causal relationship with medications and materials used during the clinical trial or with the trial itself. An adverse event may be an unfavorable or unintended sign, symptom, syndrome or disease that develops or worsens during the

clinical trial. Clinically relevant abnormal results of diagnostic procedures, including abnormal laboratory findings, are considered as adverse events.
Adverse events are categorized as "non-serious" and "serious" events.

8.12.2. Serious adverse event

A serious adverse event is an adverse event that results in any of the following outcomes:
- Death;
- Life-threatening situation;
- Hospitalization or prolongation of current hospital stay;
- Persistent or significant disability/deficiency;
- Congenital anomaly/birth defect;
- At the discretion of the Principal Researcher.

8.12.3. Recording and monitoring adverse events

Adverse events will be actively collected after the patient signs the informed consent form. Information collected should include diagnosis (based on signs and symptoms presented by the research subject), classification of event severity, start date, definition of the event causal relationship, medical practices, and event end date.

8.12.4. Definition of severity

All adverse events will be assessed (graded) regarding severity according to CTCAE version 4.0:
Grade 1 – mild adverse event
Grade 2 – moderate adverse event
Grade 3 – severe adverse event
Grade 4 – life-threatening or disabling adverse event
Grade 5 – death related to adverse event

8.12.5. Definition of drug-related adverse event

The relationship of adverse events with surgery, medications or procedures under study will be classified as follows:
- Unrelated;
- Improbable;
- Possibly related;
- Probably related;
- Definitely related.

8.13. Most Common Complications From Surgical Treatment

8.13.1. Early complications (within 30 days after surgery):

Intracavitary bleeding: originating in the surgical site or in the points of trocar insertion. Reintervention may be necessary for adequate hemostasis, with possible need for blood transfusion.

Gastrointestinal bleeding: resulting from the formation of acute ulcers of the digestive tract and/or from anastomosis sites. A new surgery may be necessary to control bleeding, with possible need for transfusion of blood components.

Anastomotic fistulas: duodeno-jejunal, entero-enteroanastomosis and gastroenteroanastomosis. The occurrence of fistulas may lead to the need for surgical treatment or even to the adoption of a conservative management. Occasionally, severe sepsis may occur, requiring prolonged hospitalizations. Surgical experience with similar procedures (bariatric surgery) indicates low rates of occurrence of this complication – 0.5/0.8% [BUCHWALD, 2004].

Gastrojejunal stenosis: occurring about 30 days after surgery. Its treatment often involves endoscopic dilatation.

Deep vein thrombosis: this event may occur in spite of the prophylaxis – use of low molecular weight heparin. The diagnosis will be made by Doppler imaging of the lower limbs. In the event of pulmonary embolism, the diagnosis will be established by CT angiography of the chest, and patients will be immediately admitted to the Intensive Care Unit.

Intestinal obstruction: this event is rarely observed in procedures performed by videolaparoscopy. The initial treatment is clinical.

Other complications: Atelectasis; Pneumonia; Pancreatitis; Hematoma at the puncture site; Vomiting, nausea.

8.13.2. Late complications: Excessive weight loss and nutritional deficiencies.

Treatment of late complications after gastric bypass consists of protein and vitamin supplementation, by enteral or parenteral route in severe cases, such as Wernicke's encephalopathy, with good results, and no cases requiring surgical reintervention have been described.

8.14. Most Common Complications From Drug Treatment

8.14.1. Metformin

The most common adverse reactions with the use of metformin are gastrointestinal disturbances such as nausea, vomiting, diarrhea, and abdominal discomfort, as well as malaise and hyperventilation. Lactic acidosis, sometimes fatal, was associated with treatment with metformin, however, almost all reported cases involved patients with contraindications to treatment or intake of excessively high doses. Occasionally, skin reactions and metallic taste may occur.

8.14.2. Pioglitazone

Although there are reports of heart failure and edema, pioglitazone appears to reduce triglyceride levels and to produce a significant increase in HDL cholesterol. There are differences in the modulation of the nuclear receptor expression of thiazolidinediones, which may explain a lower percentage of pioglitazone-related cardiovascular events compared to other drugs of the same pharmacological class.

Pioglitazone has been associated with bladder cancer and leiomyosarcoma. The PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) recorded a significant marginal increase in the incidence of bladder cancer among those treated with pioglitazone (0.5%) compared to placebo (0.2%). The authors of the study related to the PROactive trial considered the events unrelated to the drug [CHARBONNEL, 2004].

Also in the PROactive, an increase in the incidence of distal bone fractures was recorded in patients receiving pioglitazone (5.1%) compared to controls (2.5%) [CHARBONNEL, 2004].

Other drug-related reactions have been described, such as: sinusitis, headache, dental disorders, aggravated diabetes, and pharyngitis.

8.14.3. Linagliptin

Upper respiratory infections, cough, allergy, and (rarely) pancreatitis have been reported [SCHERNTHANER, 2012].

8.14.4. Liraglutide

Studies with rats have shown increased incidence of medullary thyroid carcinoma, but at doses much higher than those approved for use in human subjects. Nausea and vomiting are common, and rare cases of pancreatitis (7 cases among 4257 patients) have been described in phase 2 and 3 trials [PARKS, 2010].

8.14.5. Lantus

Hypoglycemia is the most common side effect, although it is less common in this type of slow-release insulin analog.

Allergic reactions and lipodystrophy may occur at the injection site.

8.14.6. Apidra

Hypoglycemia is the most common side effect. Allergic reactions and lipodystrophy may also occur at the injection site.

9. ETHICAL ASPECTS

9.1. ETHICAL AND REGULATORY CONSIDERATIONS

Patients will be included in the trial after formal authorization is provided in written by them by signing the informed consent form (APPENDIX 1), which will occur after adequate explanation of the nature of the project and after all questions of the patient have been

answered, according to the Brazilian National Health Council Resolution No. 196, October 10, 1996. Upon agreement to participate in the trial, the patient will periodically receive reports on the disease progress, associated with full assistance from the research team, composed of: a general surgeon, an endocrinologist, and a nutritionist.

At the end of the study, the patients will be referred back to the medical service of origin for monitoring of their disease.

9.2. REGULATIONS

This trial will be conducted in accordance with the research protocol, Good Clinical Practices, ethical principles of the Declaration of Helsinki, and the Brazilian legislation that regulates clinical research (Resolution 196/96 and complementary provisions).

The Principal Researcher will submit to the IRB written reports on the status of the clinical trial every six months. A final trial report will also be sent to the IRB after the completion of the trial or in the case of early termination of the trial, in accordance with the applicable rules. Copies of all contact with the IRB and the National Ethics Committee will be filed together with all documents related to the clinical trial.

9.3. ETHICAL APPROVAL

Before the start of the trial, the protocol will be submitted to the IRB. The trial will only start after approval of the IRB, and if applicable, of the National Ethics Committee. Any amendments to the protocol or to the informed consent form should be previously approved by the IRB prior to their implementation. The only exception is when the procedures/changes pose risks to the safety of research subjects if not performed; in this situation, the Principal Researcher will implement the changes and immediately notify the IRB.

9.4. REIMBURSEMENT OF RESEARCH SUBJECTS

Research subjects will be reimbursed for expenses related to the trial. Trial-related expenses are defined as expenses that involve transportation and meals. Research subjects will not receive any payment for participation in the clinical trial, in accordance with Brazilian law.

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Final Protocol

Title of Project:

PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

FOOTNOTES

Summary of requested changes:

Version ~~20~~21 (final) – 31out2014

(this version should have been numbered as 21, due to amendment 6 – version 20 – 11mar2014)

The protocol will amend to read:

Version 21 (final) – 31out2014

Amendment 7

Title of Project:
PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

TITLE

Summary of requested changes:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
~~João Eduardo Nunes Salles~~
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
~~Monica de Aguiar Medeiros~~
Venâncio Avancini Ferreira Alves
~~Márcio Corrêa Mancini~~
Carlos Eduardo Pompílio
Ricardo Luís Vita Nunes

The protocol will amend to read:

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Venâncio Avancini Ferreira Alves
Carlos Eduardo Pompílio
Ricardo Luís Vita Nunes

LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

Summary of requested changes:

OABq-SF	Overactive Bladder Questionaire Short Form
IPPS	International Prostate Symptoms Score

The protocol will amend to read:

OABq-SF	Overactive Bladder Questionaire Short Form
IPPS	International Prostate Symptoms Score

4.2. SECONDARY ENDPOINTS

Summary of requested changes:

7. Improvement on Quality of life (SF-36)
8. Improvement of voiding dysfunction

The protocol will amend to read:

7. Improvement on Quality of life (SF-36)
8. Improvement of voiding dysfunction

5.2. STUDY DESIGN

Summary of requested changes:

This is a prospective, open, randomized study involving ~~72~~100 patients with microvascular complications of T2DM and obesity, who will undergo gastric bypass (Roux-en-Y gastric bypass ARM A) or receive best medical treatment (ARM B, control arm).

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 12.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤12%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Those who did not reach this goal will be exclude from the study. Particular attention will be given to avoid hypoglycemia, through capillary glycemia control, especially in those patients taking insulin. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited with Oswaldo Cruz

German Hospital as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

On randomization, months 6 ~~and~~ 12, 28, 40 and 52 visits patients allocated in the surgical arm will be submitted to elastography ARFI (Acoustic Radiation Force Impulse). The method quantifies the degree of fibrosis with high accuracy, with the advantages of being coupled to a conventional ultrasound equipment (Siemens S2000), allowing the visualization of the liver and determining the liver segment to be analyzed.

The protocol will amend to read:

This is a prospective, open, randomized study involving 100 patients with microvascular complications of T2DM and obesity, who will undergo gastric bypass (Roux-en-Y gastric bypass ARM A) or receive best medical treatment (ARM B, control arm).

Fifty obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

On randomization, months 6,12, 28,40 and 52 visits patients allocated in the surgical arm will be submitted to elastography ARFI (Acoustic Radiation Force Impulse). The method quantifies the degree of fibrosis with high accuracy, with the advantages of being coupled to a conventional ultrasound equipment (Siemens S2000), allowing the visualization of the liver and determining the liver segment to be analyzed.

5.2. STUDY DESIGN

Summary of requested changes:

At randomization visits and months 6, 12, ~~and~~ 24, 36, 48 and 60, 3 to 5 mL of urine will be collected in both arms of the study. In these samples, DNA sequencing will be investigated for correlation of urinary metabolites with the success of treatments in relation to the control of microvascular disease secondary to diabetes. The samples will be analyzed at Santa Casa de Misericórdia – São Paulo/Brazil and will be stored until the end of the study. This collection will also be included in the Informed Consent Form, that will be signed by research ~~subject~~participant.

The protocol will amend to read:

In the randomization and months 6,12,24,36,48 and 60 visits will be collected 3 to 5 ml of urine, in both study arms. In these samples, DNA sequencing will be searched for correlation of urinary metabolites with the success of treatments in the control of secondary microvascular disease to diabetes. The samples will be analyzed at the Santa Casa de Misericordia - São Paulo and will be stored until the end of the study. This collection also will be included in the Informed Consent Form that will be signed by the research participant.

5.2. STUDY DESIGN

Summary of requested changes:

The control group will include ~~thirty-six~~ fifty patients with microvascular complications of T2DM who will receive the best clinical treatment available, which will consist of metformin, glitazones, incretin therapy (DPP4 inhibitor and GLP-1 analogs) and insulin, if necessary [DeFRONZO, 2009]. In addition, other comorbidities, such as hypertension and dyslipidemia, will be compensated according to ADA recommendations [ADA, 2012].

The protocol will amend to read:

The control group will include fifty patients with microvascular complications of T2DM who will receive the best clinical treatment available, which will consist of metformin, glitazones, incretin therapy (DPP4 inhibitor and GLP-1 analogs) and insulin, if necessary [DeFRONZO, 2009]. In addition, other comorbidities, such as hypertension and dyslipidemia, will be compensated according to ADA recommendations [ADA, 2012].

6.5. RECRUITMENT AND FOLLOW UP

Summary of requested changes:

The recruitment period will last for ~~6~~ 2 years (24 months) from the beginning of the study (initiation visit), and follow-up will proceed for ~~24~~ 60 months after participant inclusion in the study.

The protocol will amend to read:

The recruitment period will last for 2 years (24 months) from the beginning of the study (initiation visit), and follow-up will proceed for 60 months after participant inclusion in the study.

6.6.2. SAMPLE SIZE CALCULATION

Summary of requested changes:

Thus, considering the data in the literature and unpublished data of the group, as well as a rate of loss to follow-up of 15% for both groups and a two-tailed type I error (α) of 5%, ~~some scenarios are demonstrated,~~ the final scenario is:

Statistical power of 90%

The final scenario underlying the increase of the segment and following conjecture a loss of 38%, assuming the following probabilities:
Pc = 10% (probability of achieving the primary endpoint in the clinical group)
Pc = 50% (probability of achieving the primary endpoint in the surgical group)

~~Statistical power of 80%~~

~~Scenario 1~~
~~Pc = 10% (probability of achieving the primary endpoint in the clinical group)~~
~~Pc = 50% (probability of achieving the primary endpoint in the surgical group)~~
~~Number of research subjects in each arm = 29~~
~~Total number of patients in the study = 58~~

~~Scenario 2~~
~~Pc = 5% (probability of achieving the primary endpoint in the clinical group)~~
~~Pc = 60% (probability of achieving the primary endpoint in the surgical group)~~
~~Number of research subjects in each arm = 16~~
~~Total number of patients in the study = 32~~

~~Scenario 3~~
~~Pc = 5% (probability of achieving the primary endpoint in the clinical group)~~
~~Pc = 70% (probability of achieving the primary endpoint in the surgical group)~~
~~Number of research subjects in each arm = 13~~
~~Total number of patients in the study = 26~~

~~The three previous scenarios are less conservative (considering Scenario 1 as the most conservative), with less chance of detecting a biological and statistically significant effect, worse with greater differences between Ps and Pc.~~

~~Statistical power of 90%~~

~~Scenario 4~~
~~Pc = 10% (probability of achieving the primary endpoint in the clinical group)~~
~~Pc = 50% (probability of achieving the primary endpoint in the surgical group)~~
~~Number of research subjects in each arm = 36~~
~~Total number of patients in the study = 72~~

~~Scenario 5~~
~~Pc = 5% (probability of achieving the primary endpoint in the clinical group)~~
~~Pc = 60% (probability of achieving the primary endpoint in the surgical group)~~
~~Number of research subjects in each arm = 20~~
~~Total number of patients in the study = 40~~

~~Scenario 6~~
~~Pc = 5% (probability of achieving the primary endpoint in the clinical group)~~
~~Pc = 70% (probability of achieving the primary endpoint in the surgical group)~~
~~Number of research subjects in each arm = 15~~
~~Total number of patients in the study = 30~~

~~These are more conservative scenarios (4, 5 and 6), com increased chance of detecting significant differences, worse with greater differences between Ps and Pc, as like in the previous scenarios.~~

As the best option to answer the question of this protocol, and assuming a conservative profile as a guarantee to obtain reliable results, we chose the conditions Ps and 50% PC 10%, with a statistical power of 90% (~~scenario 4~~, final scenario). These parameters show an optimal number of research subjects in each arm of ~~36~~50 patients, reaching a total of ~~72~~100 patients in the study.

The protocol will amend to read:

Thus, considering the data in the literature and unpublished data of the group, as well as a rate of loss to follow-up of 15% for both groups and a two-tailed type I error (α) of 5%, the final scenario is:

Statistical power of 90%

The final scenario underlying the increase of the segment and following conjecture a loss of 38%, assuming the following probabilities:
Pc = 10% (probability of achieving the primary endpoint in the clinical group)
Pc = 50% (probability of achieving the primary endpoint in the surgical group)

As the best option to answer the question of this protocol, and assuming a conservative profile as a guarantee to obtain reliable results, we chose the conditions Ps and 50% PC 10%, with a statistical power of 90% (final stage). These parameters show an optimal number of research subjects in each arm of 50 patients, reaching a total of 100 patients in the study.

7.1. SELECTION/SCREENING

Summary of requested changes:

- Quality of life assessment using the SF-36;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Baseline Visit;
- Laboratory tests as described in the Flowchart of the Study and in Assessments and Tests;

The protocol will amend to read:

- Quality of life assessment using the SF-36;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Baseline Visit;
- Laboratory tests as described in the Flowchart of the Study and in Assessments and Tests;

7.2. BASELINE VISIT

Summary of requested changes:

- Urine sample collection for DNA sequencing;
- Collect voiding diary to control urinary habit, provided in the Screening visit.

The protocol will amend to read:

- Urine sample collection for DNA sequencing;
- Collect voiding diary to control urinary habit, provided in the Screening visit.

7.4.3. VISITS – WEEK 4, MONTH 3, MONTH 6, MONTH 9, MONTH 15 AND MONTH 21

Summary of requested changes:

- Assessment of capillary glucose, for both arms;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Monh 6 (only on Month 3);
- Dispensing of drugs and materials needed for the subject until next visit. All patients in the surgical arm will use multivitamin supplements daily until the end of the study.

The protocol will amend to read:

- Assessment of capillary glucose, for both arms;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Monh 6 (only on Month 3);
- Dispensing of drugs and materials needed for the subject until next visit. All patients in the surgical arm will use multivitamin supplements daily until the end of the study.

7.4.4. VISITS – MONTH 6 AND MONTH 18

Summary of requested changes:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF (only on Month 6);

- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 12 Visit (only on Month 6);
- Urine sample collection for DNA sequencing;

The protocol will amend to read:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF (only on Month 6);
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 12 Visit (only on Month 6);
- Urine sample collection for DNA sequencing;

7.4.5. MONTH 12 AND MONTH 24 VISITS

Summary of requested changes:

- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 24 and Month 36 Visit, consecutively;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit ~~(Month 12)~~;

The protocol will amend to read:

- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 24 and Month 36 Visit, consecutively;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake.

During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

7.4.6. MONTH 28 VISIT

Summary of requested changes:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Assessment of capillary glucose;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

The protocol will amend to read:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;

- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Assessment of capillary glucose;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

7.4.7. MONTH 32, MONTH 44 AND MONTH 56 VISITS

Summary of requested changes:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Assessment of capillary glucose;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

The protocol will amend to read:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;

- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Assessment of capillary glucose;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

7.4.8. MONTH 36

Summary of requested changes:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 48;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

The protocol will amend to read:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 48;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

7.4.9. MONTH 40 E MONTH 52 VISITS

Summary of requested changes:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;

- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

The protocol will amend to read:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit.

7.4.10. MONTH 48 AND MONTH 60

Summary of requested changes:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 60;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit (only on Month 48).

The protocol will amend to read:

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;

- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose;
- Voiding dysfunction assessment using the I-PSS and OAB-qSF;
- Guidance and dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days. Return of the voiding diary filled on the day scheduled for Month 60;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of materials and drugs needed until the next visit (only on Month 48).

7.5. PROCEDURES FLOWCHART

Summary of requested changes:

	Year 1										Year 2				Year 3			Year 4			Year 5		
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24	M28	M32	M36	M40	M44	M48	M52	M56	M60
Informed consent	X																						
Anamnesis	X																						
Inclusion/exclusion criteria	X	X																					
Medical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X																					
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X							X		X		X		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nerve conduction studies	X							X		X		X		X	X	X	X	X	X	X	X	X	X
Nutritional assessment	X				X	X	X	X	X	X	X	X	X	X	X		X	X		X	X		X
SF-36 administration	X									X				X			X			X			X
I-PSS assessment	X									X				X			X			X			X
OAB-qSF assessment	X									X				X			X			X			X
Dispensation - voiding diary	X						X	X		X				X			X			X			X
Serum pregnancy test	X																						
Elastrography*		X						X		X					X			X			X		
Complete blood count	X									X		X		X			X			X			X
AST/ALT	X									X				X			X			X			X
Amylase	X							X***				X***			X			X			X		
Sodium and potassium	X																						
Urea and creatinine	X						X	X		X		X		X	X	X	X	X	X	X	X	X	X
Coagulogram (PT and APTT)	X																						
Lipid profile	X						X	X		X		X		X	X		X	X		X	X		X
Fasting glycemia				X	X	X	X		X		X		X		X	X	X	X	X	X	X	X	X
HbA1c	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Microalbuminuria alone	X							X		X		X		X	X		X	X		X	X		X
Urinary creatinine	X							X		X		X		X	X		X	X		X	X		X
Iron and ferritin	X							X		X		X		X			X			X			X
HOMA	X							X		X		X		X			X			X			X

	Year 1										Year 2				Year 3			Year 4			Year 5		
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24	M28	M32	M36	M40	M44	M48	M52	M56	M60
Meal tolerance test	X							X		X		X		X	X		X	X		X	X		X
Ionazable calcium	X							X		X		X		X			X			X			X
PTH	X							X		X		X		X			X			X			X
Vit D (25 OH)	X							X		X		X		X			X			X			X
Vit A	X							X				X		X			X			X			X
Folic acid	X							X						X			X			X			X
Vit B1	X							X				X		X			X			X			X
Vit B12	X							X				X		X			X			X			X
Anti-GAD	X																						
Urine I	X									X				X			X			X			X
RCP			X*				X*	X*		X*		X*		X*	X*		X*	X*		X*	X*		X*
Urine culture																							
Echocardiogram	X																						X
Urine for DNA sample		X						X		X				X			X			X			X
PSA	X																						
Anti-HIV	X																						
Chest x-ray	X																						
Abdominal ultrasound	X																						
Electrocardiography	X																						
Upper digestive endoscopy		X*																					
Color retinography		X						X		X		X		X									
Angiofluoresceinography		X						X		X		X		X									
Refraction test		X						X		X		X		X									
Vital signs	X		X	X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X			X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																						
Drug dispensing	X		X	X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Glucose assessment					X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gastric bypass			X																				
Hospitalization			X																				

* Applicable for patients randomized to the intervention group (surgical procedure).
** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days prior to randomization visit (baseline), for patients in the clinical arm and from the surgery for patients in the surgical arm.
*** Applicable for patients randomized to the clinical group

The protocol will amend to read:

	Year 1										Year 2				Year 3			Year 4			Year 5		
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24	M28	M32	M36	M40	M44	M48	M52	M56	M60
Informed consent	X																						
Anamnesis	X																						
Inclusion/exclusion criteria	X	X																					
Medical examination	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X																					
Concomitant medications	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Physical examination	X							X		X		X		X	X	X	X	X	X	X	X	X	X
Adverse events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nerve conduction studies	X							X		X		X		X	X	X	X	X	X	X	X	X	X
Nutritional assessment	X				X	X	X	X	X	X	X	X	X	X	X		X	X		X	X		X
SF-36 administration	X									X				X			X			X			X
I-PSS assessment	X									X				X			X			X			X
OAB-qSF assessment	X									X				X			X			X			X
Dispensation - voiding diary	X						X	X		X				X			X			X			X
Serum pregnancy test	X																						
Elastrography*		X						X		X					X			X			X		
Complete blood count	X									X		X		X			X			X			X
AST/ALT	X									X				X			X			X			X
Amylase	X							X***				X***			X			X			X		
Sodium and potassium	X																						
Urea and creatinine	X						X	X		X		X		X	X	X	X	X	X	X	X	X	X
Coagulogram (PT and APTT)	X																						
Lipid profile	X						X	X		X		X		X	X		X	X		X	X		X
Fasting glycemia				X	X	X	X		X		X		X		X	X	X	X	X	X	X	X	X
HbA1c	X						X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Microalbuminuria alone	X							X		X		X		X	X		X	X		X	X		X
Urinary creatinine	X							X		X		X		X	X		X	X		X	X		X
Iron and ferritin	X							X		X		X		X			X			X			X
HOMA	X							X		X		X		X			X			X			X

	Year 1										Year 2				Year 3			Year 4			Year 5		
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24	M28	M32	M36	M40	M44	M48	M52	M56	M60
Meal tolerance test	X							X		X		X		X	X		X	X		X	X		X
Ionazable calcium	X							X		X		X		X			X			X			X
PTH	X							X		X		X		X			X			X			X
Vit D (25 OH)	X							X		X		X		X			X			X			X
Vit A	X							X				X		X			X			X			X
Folic acid	X							X						X			X			X			X
Vit B1	X							X				X		X			X			X			X
Vit B12	X							X				X		X			X			X			X
Anti-GAD	X																						
Urine I	X									X				X			X			X			X
RCP			X*				X*	X*		X*		X*		X*	X*		X*	X*		X*	X*		X*
Urine culture																							
Echocardiogram	X																						X
Urine for DNA sample		X						X		X				X			X			X			X
PSA	X																						
Anti-HIV	X																						
Chest x-ray	X																						
Abdominal ultrasound	X																						
Electrocardiography	X																						
Upper digestive endoscopy		X*																					
Color retinography		X						X		X		X		X									
Angiofluoresceinography		X						X		X		X		X									
Refraction test		X						X		X		X		X									
Vital signs	X		X	X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Weight	X			X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Height	X																						
Drug dispensing	X		X	X	X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Glucose assessment					X*	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Gastric bypass			X																				
Hospitalization			X																				

* Applicable for patients randomized to the intervention group (surgical procedure).
** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days prior to randomization visit (baseline), for patients in the clinical arm and from the surgery for patients in the surgical arm.
*** Applicable for patients randomized to the clinical group

7.6. ASSESSMENTS AND TESTS

Summary of requested changes:

Only retinography and elastography will be performed in outpatient clinics. These were previously audited in relation to diagnostic method and specificity study.

The protocol will amend to read:

Only retinography and elastography will be performed in outpatient clinics. These were previously audited in relation to diagnostic method and specificity study.

7.6.10. ESTIMATE OF MEDICATIONS AND CAPPILARY GLYCEMIA REAGENT STRIPS USED

Summary of requested changes:

Product	Surgical group (n=50)	Clinical group (n= 50)
Metformin 1g	4200 cp/month	4200 cp/month
Actos 30 mg	420 cp/month	630 cp/month
Linagliptin 5 mg	420 cp/month	630 cp/month
Liraglutide 6.0 mg/mL	~~28~~5 pens/month	~~28~~100 pens/month
Lantus	10500 IU/month	21000 IU/month
Apidra	1300 IU/month	5000 IU/month
Centrum	2100 cp/month	
Reagent strips	~~31~~4000/month	~~31~~4000/month

The protocol will amend to read:

Product	Surgical group (n=50)	Clinical group (n= 50)
Metformin 1g	4200 cp/month	4200 cp/month
Actos 30 mg	420 cp/month	630 cp/month
Linagliptin 5 mg	420 cp/month	630 cp/month
Liraglutide 6.0 mg/mL	5 pens/month	100 pens/month
Lantus	10500 IU/month	21000 IU/month
Apidra	1300 IU/month	5000 IU/month
Centrum	2100 cp/month	
Reagent strips	4000/month	4000/month

7.6.11. EVALUATION OF VOIDING DYSFUNCTION

Summary of requested changes:

In addition to the microvascular complications such as nephropathy, retinopathy and neuropathy, diabetic bladder dysfunction is described also known as diabetic cistopatia

consisting of changes dependent on the retention time and bladder emptying, is the most important complication of lower urinary tract due to T2DM and correlated with oxidative stress caused by this [KEPABSCI, 2007; DANESHGARI 2009]. Still, there is evidence that weight loss in obese induced by bariatric surgery, promotes significant improvement in symptoms of lower urinary tract, such as urinary incontinence, stress or urgency, both in women as in men. Thus, we propose a voiding evaluation of these patients through two questionnaires and record in voiding diary for control of urinary habits.

Voiding dysfunction evaluation as a complication of T2DM in mild obese patients will be assessed by applying two questionnaires and reporting in a voiding diary to control urinary habit, as described below:

- Application of questionnaires IPSS (International Prostate Sympton Score) and OAB-q (Overactive Bladder Questionnaire short form), both with validation for the Portuguese language and self-applicable. Although originally intended for evaluation only of prostatic symptoms in men, the IPSS has been applied to women, as the clinical signs of prostate problems (symptoms of lower urinary tract) may also occur due to other common comorbidities to both sexes [OKAMURA, 2009; HSIAO, 2013; CHOI, 2014].

Questionnaires will be applied at baseline and after 6, 12, 24, 36, 48 and 60 months of intervention. Dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days for the next visit (month 12, 24, 36, 48 and 60)

The comparison will be made between the initial time and the subsequent.

The protocol will amend to read:

In addition to the microvascular complications such as nephropathy, retinopathy and neuropathy, diabetic bladder dysfunction is described also known as diabetic cistopatia consisting of changes dependent on the retention time and bladder emptying, is the most important complication of lower urinary tract due to T2DM and correlated with oxidative stress caused by this [KEPABSCI, 2007; DANESHGARI 2009]. Still, there is evidence that weight loss in obese induced by bariatric surgery, promotes significant improvement in symptoms of lower urinary tract, such as urinary incontinence, stress or urgency, both in women as in men. Thus, we propose a voiding evaluation of these patients through two questionnaires and record in voiding diary for control of urinary habits.

Voiding dysfunction evaluation as a complication of T2DM in mild obese patients will be assessed by applying two questionnaires and reporting in a voiding diary to control urinary habit, as described below:

- Application of questionnaires IPSS (International Prostate Sympton Score) and OAB-q (Overactive Bladder Questionnaire short form), both with validation for the Portuguese language and self-applicable. Although originally intended for evaluation only of prostatic symptoms in men, the IPSS has been applied to women, as the clinical signs of prostate problems (symptoms of lower urinary tract) may also occur due to other common comorbidities to both sexes [OKAMURA, 2009; HSIAO, 2013; CHOI, 2014].

Questionnaires will be applied at baseline and after 6, 12, 24, 36, 48 and 60 months of intervention. Dispensation of voiding diary to control urinary habit to be completed in three non-consecutive days for the next visit (month 12, 24, 36, 48 and 60)

The comparison will be made between the initial time and the subsequent.

Amendment 6

Title of Project:

PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

7. PROCEDURES FLOWCHART

Summary of requested changes:

Assessments	Screen **	Basel	Surg*	1st to 3rd PO	W 1	W 4	M 3	M 6	M 9	M 12	M15	M 18	M 21	M 24
Complete blood count	X									X		X		X
AST/ALT	X									X				X
Urea and creatinine	X						X	X	~~X~~	X	~~X~~	X	~~X~~	X
Urinary creatinine	X							X		X		X		X
Iron and ferritin	X							X		X		~~X~~		X
Vit A	X							X		~~X~~		X		X
Folic acid	X							X		~~X~~				X
Vit B1	X							X		~~X~~		X		X
Vit B12	X							X		~~X~~		X		X
Anti- GAD	X													

7. PROCEDURES FLOWCHART

The protocol will amend to read:

Assessments	Screen **	Basel	Surg*	1st to 3rd PO	W 1	W 4	M 3	M 6	M 9	M 12	M15	M 18	M 21	M 24
Complete blood count	X									X		X		X
AST/ALT	X									X				X
Urea and creatinine	X						X	X		X		X		X
Urinary creatinine	X							X		X		X		X
Iron and ferritin	X							X		X				X
Vit A	X							X				X		X
Folic acid	X							X						X
Vit B1	X							X				X		X
Vit B12	X							X				X		X
Anti- GAD	X													

Amendment 5

Title of Project:
PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

TITLE

Summary of requested changes:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
João Eduardo Salles Nunes
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Monica de Aguiar Medeiros
Venâncio Avancini Ferreira Alves
Márcio Correa Mancini

The protocol will amend to read:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
João Eduardo Salles Nunes
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Monica de Aguiar Medeiros
Venâncio Avancini Ferreira Alves
Márcio Correa Mancini

1. LIST OF ABREVIATIONS AND DEFINITIONS OF TERMS

Summary of requested changes:

CONEP	National Research Ethics Committee
CRP	C Reative Protein
DCCT	Diabetes Control and Complications Trial

The protocol will amend to read:

CONEP	National Research Ethics Committee
CRP	C Reative Protein
DCCT	Diabetes Control and Complications Trial

2. INTRODUCTION

Summary of requested changes:

Table 1. Antidiabetic Drugs Approved in the United States

Drug	Route of Administration	Market Release Date	% of reduction in HbA1c when used as a monotherapy
Thiazolidinedione	Oral	1999	0.8-1.0

The protocol will amend to read:

Table 1. Antidiabetic Drugs Approved in the United States

Drug	Route of Administration	Market Release Date	% of reduction in HbA1c when used as a monotherapy
Thiazolidinedione	Oral	1999	0.8-1.0

6.1. STUDY DESIGN

Summary of requested changes:

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 1~~0~~2.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤1~~0~~2%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011].

The protocol will amend to read:

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 12.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤12%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011].

7.2. BASELINE VISIT

Summary of requested changes:

- Subjects randomly assigned to the intervention group (surgery) who present HbA1c ≥ 10~~0~~2,0% will be clinically treated during 3 (three) months and will be reevaluated in a new baseline visit. If during this new baseline the subject still presents HbA1c ≥ 12,0%, he will be excluded from the study.

The protocol will amend to read:

- Subjects randomly assigned to the intervention group (surgery) who present HbA1c ≥ 12,0% will be clinically treated during 3 (three) months and will be reevaluated in a new baseline visit. If during this new baseline the subject still presents HbA1c ≥ 12,0%, he will be excluded from the study.

7.2. BASELINE VISIT

Summary of requested changes:

- Upper digestive endoscopy only for patients randomized to surgery group, including H. pylori infection investigation and biopsy only if necessary;
- Elastography;

The protocol will amend to read:

- Upper digestive endoscopy only for patients randomized to surgery group, including H. pylori infection investigation and biopsy only if necessary;
- Elastography;

- Provide information about surgical procedure and drug treatment for subjects randomized to the intervention group (surgery procedure);

7.3. SURGERY

Summary of requested changes:

- Hepatic biopsy during surgery: the extracted fragment will be forwarded to pathological analysis, as described in the item 6.1;
- Laboratorial exam collection: RCP;
~~- Adipose tissue extraction during surgical procedure, as described in item 6.1.~~
- Medical examination of the subjects' overall clinical status;

The protocol will amend to read:

- Hepatic biopsy during surgery: the extracted fragment will be forwarded to pathological analysis, as described in the item 6.1;
- Laboratorial exam collection: RCP;

- Medical examination of the subjects' overall clinical status;

7.4.1. FIRST TO THIRD POSTOPERATIVE DAY (SURGICAL ARM PATIENTS)

Summary of requested changes:

- Dispensing of drugs and materials needed for the subject until next visit~~;~~. All patients will use Ranitidine hydrochloride (150mg/day).

The protocol will amend to read:

- Dispensing of drugs and materials needed for the subject until next visit~~;~~. All patients will use Ranitidine hydrochloride (150mg/day).

7.4.3. VISIT – WEEK 4, MONTH 3, MONTH 9, MONTH 15 AND MONTH 21

Summary of requested changes:

- Dispensing of drugs and materials needed for the subject until next visit~~;~~. All patients in the surgical arm will use multivitamin supplements daily until the end of the study.

The protocol will amend to read:

- Dispensing of drugs and materials needed for the subject until next visit. All patients in the surgical arm will use multivitamin supplements daily until the end of the study.

TITLE

Summary of requested changes:

	Year 1									Year 2			
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Amylase	X							X***				X***		
Fasting glycemia				X	X*	X	X		X		X		X	
Microalbuminuria (isolated sample)	X							X		X		X		X
RCP			X				X	X		X		X		X
Vital signs	X		X	X	X*	X	X	X	X	X	X	X	X	X
Drug dispensing	X		X	X	X*	X	X	X	X	X	X	X	X	X
Glucose assessment					X*	X	X	X	X	X	X	X	X	X

* Applicable for patients randomized to the intervention group (surgical procedure).
** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days prior to randomization visit (baseline), for patients in the clinical arm and from the surgery for patients in the surgical arm.
*** Applicable for patients randomized to the clinical group

The protocol will amend to read:

	Year 1									Year 2			
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Amylase	X							X***				X***		
Fasting glycemia				X	X*	X	X		X		X		X	
Microalbuminuria (isolated sample)	X							X		X		X		X
RCP			X				X	X		X		X		X
Vital signs	X		X	X	X*	X	X	X	X	X	X	X	X	X
Drug dispensing	X		X	X	X*	X	X	X	X	X	X	X	X	X
Glucose assessment					X*	X	X	X	X	X	X	X	X	X

* Applicable for patients randomized to the intervention group (surgical procedure).
** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days prior to randomization visit (baseline), for patients in the clinical arm and from the surgery for patients in the surgical arm.
*** Applicable for patients randomized to the clinical group

Amendment 4

Title of Project:
PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

TITLE

Summary of requested changes:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
João Eduardo Salles Nunes
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Monica de Aguiar Medeiros
Venâncio Avancini Ferreira Alves

The protocol will amend to read:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
João Eduardo Salles Nunes
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Monica de Aguiar Medeiros
Venâncio Avancini Ferreira Alves

6.1. STUDY DESIGN

Summary of requested changes:

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with

the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Those who did not reach this goal will be exclude from the study. Particular attention will be given to avoid hypoglycemia, through capillary glycemia control, especially in those patients taking insulin. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited with Oswaldo Cruz German Hospital as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

On randomization, months 6 and 12 visits patients allocated in the surgical arm will be submitted to elastography ARFI (Acoustic Radiation Force Impulse). The method quantifies the degree of fibrosis with high accuracy, with the advantages of being coupled to a conventional ultrasound equipment (Siemens S2000), allowing the visualization of the liver and determining the liver segment to be analyzed.

The literature, including the first national study pilot shows high accuracy in quantifying the degree of fibrosis compared to liver biopsy in hepatopathy, nonalcoholic steatohepattis, steatohepatitis and other numerous liver diseases. [Junior, 2012; Friedich-Rust, 2012; Palmeri, 2011; Boursier, 2010]

The method has been used routinely in many countries and has been introduced in our country, in order to reduce the number of liver biopsies. The elastography tests will be carried out in Schmillevitch Diagnostics Center - São Paulo, by a single operator.

Compared to the diagnostic method mentioned above, during.~~During~~ the surgery, liver biopsy will be performed in order to quantify the degree of hepatic steatosis~~, as well as the extraction of adipose tissue to quantify the glucocorticoid and inflammatory activity. Approximately 0.5 to 1cm of visceral fat – large omentum (producing inflammation and cytokines), 0.5 to 1cm of subcutaneous fat (producing anti-inflammatory adipokines) and 0.5 to 1cm of muscle tissue (insulin-sensitive organ and major consumer of glucose). Through these fatty fragments, it will be possible to study the inflammatory status of the tissues by quantifying the local NFkB gene (inflammatory pathway). Local tissue inflammation may be both a cause of axillary inflammation and cause of topical hyperactivation of glucocorticoid activity. These fatty fragments will be stores at the Santa Casa de Misericórdia – São Paulo/Brazil and will be used during the study period, after informed consent from MOMS patients.~~

The protocol will amend to read:

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Those who did not reach this goal will be exclude from the study. Particular attention will be given to avoid hypoglycemia, through capillary glycemia control, especially in those patients taking insulin.

Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited with Oswaldo Cruz German Hospital as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

On randomization, months 6 and 12 visits patients allocated in the surgical arm will be submitted to elastography ARFI (Acoustic Radiation Force Impulse). The method quantifies the degree of fibrosis with high accuracy, with the advantages of being coupled to a conventional ultrasound equipment (Siemens S2000), allowing the visualization of the liver and determining the liver segment to be analyzed.

The literature, including the first national study pilot shows high accuracy in quantifying the degree of fibrosis compared to liver biopsy in hepatopathy, nonalcoholic steatohepattis, steatohepatitis and other numerous liver diseases. [Junior, 2012; Friedich-Rust, 2012; Palmeri, 2011; Boursier, 2010]

The method has been used routinely in many countries and has been introduced in our country, in order to reduce the number of liver biopsies. The elastography tests will be carried out in Schmillevitch Diagnostics Center - São Paulo, by a single operator.

Compared to the diagnostic method mentioned above, during the surgery, liver biopsy will be performed in order to quantify the degree of hepatic steatosis.

7.5. PROCEDURES FLOWCHART

Summary of requested changes:

	Year 1										Year 2			
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Elastrography*		X						X		X				

The protocol will amend to read:

	Year 1										Year 2			
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Elastrography*		X						X		X				

10. REFERENCES

Summary of requested changes:

Boursier J; Isselin G; Fouchard-Hubert I; Oberti F; Dib N; Lebigot J; Bertrais S; Gallois Y; Cales P; Aubé C. Acoustic radiation force impulse: a new ultrassonagraphic techonology for the widespread nonivasive diagnosis of liver fibrosis. European Journal of gastroenterology and Hepatology.2010 22(9):1074-84.

Junior RGS; Vieira A; Schmillevitch J; Szutan LA. ARFI Elastrography for Liver fibrosis assessment in cronic hepatitis-pilot study. Annlas of Hepatology. 2012;11(5):751-805.

Palmeri MI; Wang MH; Rouze NC; Abdelmalek MF; Guy CD; Moser B; Diehl AM; Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force – based shear stiffness in patients with nonalcoholic fatty liver disease. Journal of Hepatology. 2012;55(3):666-72

The protocol will amend to read:

Boursier J; Isselin G; Fouchard-Hubert I; Oberti F; Dib N; Lebigot J; Bertrais S; Gallois Y; Cales P; Aubé C. Acoustic radiation force impulse: a new ultrassonagraphic techonology for the widespread nonivasive diagnosis of liver fibrosis. European Journal of gastroenterology and Hepatology.2010 22(9):1074-84.

Junior RGS; Vieira A; Schmillevitch J; Szutan LA. ARFI Elastrography for Liver fibrosis assessment in cronic hepatitis-pilot study. Annlas of Hepatology. 2012;11(5):751-805.

Palmeri MI; Wang MH; Rouze NC; Abdelmalek MF; Guy CD; Moser B; Diehl AM; Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force – based shear stiffness in patients with nonalcoholic fatty liver disease. Journal of Hepatology. 2012;55(3):666-72

Amendment 3

Title of Project:
PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

6.1. STUDY DESIGN

Summary of requested changes:

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Those who did not reach this goal will be exclude from the study. Particular attention will be given to avoid hypoglycemia, through capillary glycemia control, especially in those patients taking insulin. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited with Oswaldo Cruz German Hospital as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

During the surgery, hepatic biopsy will be performed in order to quantify the degree of hepatic steatosis, as well as the extraction of adipose tissue to quantify the glucocorticoid and inflammatory activity. Approximately 0.5 to 1cm of visceral fat – large omentum (producing inflammation and cytokines), 0.5 to 1cm of subcutaneous fat (producing anti-inflammatory adipokines) and 0.5 to 1cm of muscle tissue (insulin-sensitive organ and major consumer of glucose). Through these fatty fragments, it will be possible to study the inflammatory status of the tissues by quantifying the local NFkB gene (inflammatory pathway). Local tissue inflammation may be both a cause of axillary inflammation and cause of topical hyperactivation of glucocorticoid activity. These fatty fragments will be stores at the Santa Casa de Misericórdia – São Paulo/Brazil and will be used during the study period, after informed consent from MOMS patients.

At randomization visits and months 6, 12 and 24, 3to5 mL of urine will be collected in both arms of the study. In these samples, DNA sequencing will be investigated for correlation of urinary metabolites with the success of treatments in relation to the control of microvascular disease secondary to diabetes. The samples will be analyzed at Santa Casa de Misericórdia – São Paulo/Brazil and will be stored until the end of the study. This

collection will also be included in the Informed Consent Form, that will be signed by research subject.

Regarding medication to be used, if there is no contraindication, metformin will be maintained in the postoperative period while fasting glycemia is above 100 mg/dL. Anti-antihypertensive drugs and medications for dyslipidemia will be maintained in the postoperative period, unless there are any contraindications. A supplement of micronutrients (vitamins and mineral salts) will be prescribed to all patients who undergo surgery. Patients allocated to the control group will receive the same supplementation if necessary.

The protocol will amend to read:

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0% who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Those who did not reach this goal will be exclude from the study. Particular attention will be given to avoid hypoglycemia, through capillary glycemia control, especially in those patients taking insulin. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited with Oswaldo Cruz German Hospital as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

During the surgery, hepatic biopsy will be performed in order to quantify the degree of hepatic steatosis, as well as the extraction of adipose tissue to quantify the glucocorticoid and inflammatory activity. Approximately 0.5 to 1cm of visceral fat – large omentum (producing inflammation and cytokines), 0.5 to 1cm of subcutaneous fat (producing anti-inflammatory adipokines) and 0.5 to 1cm of muscle tissue (insulin-sensitive organ and major consumer of glucose). Through these fatty fragments, it will be possible to study the inflammatory status of the tissues by quantifying the local NFkB gene (inflammatory pathway). Local tissue inflammation may be both a cause of axillary inflammation and cause of topical hyperactivation of glucocorticoid activity. These fatty fragments will be stores at the Santa Casa de Misericórdia – São Paulo/Brazil and will be used during the study period, after informed consent from MOMS patients.

At randomization visits and months 6, 12 and 24, 3to5 mL of urine will be collected in both arms of the study. In these samples, DNA sequencing will be investigated for correlation of urinary metabolites with the success of treatments in relation to the control of microvascular disease secondary to diabetes. The samples will be analyzed at Santa Casa de Misericórdia – São Paulo/Brazil and will be stored until the end of the study. This collection will also be included in the Informed Consent Form, that will be signed by research subject.

Regarding medication to be used, if there is no contraindication, metformin will be maintained in the postoperative period while fasting glycemia is above 100 mg/dL. Anti-antihypertensive drugs and medications for dyslipidemia will be maintained in the postoperative period, unless there are any contraindications. A supplement of micronutrients (vitamins and mineral salts) will be prescribed to all patients who undergo surgery. Patients allocated to the control group will receive the same supplementation if necessary.

6.6.3. RANDOMIZATION AND ALLOCATION OF PATIENTS

Summary of requested changes:

Concealed allocation will be ensured through the use of ~~opaque and sealed envelopes (SNOSE approach), numbered sequentially. The envelope will be opened by the physician/investigator in the first patient assessment, where allocation order is irreversible.~~ random IDs (group ID). These IDs will be used to name the patients, so that does not contain personal information. So that, through a randomization, clinical research coordinator will send to the statistical gender identification number (male/female). The statistical returns only referencing the group that patients were allocated (Group 1: surgery, Group 0: clinical).

The protocol will amend to read:

Concealed allocation will be ensured through the use of random IDs (group ID). These IDs will be used to name the patients, so that does not contain personal information. So that, through a randomization, clinical research coordinator will send to the statistical gender identification number (male/female). The statistical returns only referencing the group that patients were allocated (Group 1: surgery, Group 0: clinical).

7.1. SELECTION/SCREENING

Summary of requested changes:

- ~~Upper digestive endoscopy, including H. pylori infection investigation and biopsy only if necessary; only for patients randomized to surgery group.~~

The protocol will amend to read:

7.2. BASELINE VISIT

- Upper digestive endoscopy only for patients randomized to surgery group;

7.2. BASELINE VISIT

Summary of requested changes:

- Provide information about drug treatment for subjects randomized to the control group.
- Urine sample collection for DNA sequencing;

The protocol will amend to read:

- Provide information about drug treatment for subjects randomized to the control group;
- Urine sample collection for DNA sequencing.

7.3. SURGERY

Summary of requested changes:

- Gastric bypass as described in the item 6.2 (Surgery plan);
- Hepatic biopsy during surgery: the extracted fragment will be forwarded to pathological analysis, as described in the item 6.1;
- Adipose tissue extraction during surgical procedure, as described in item 6.1.
- Medical examination of the subjects' overall clinical status;

The protocol will amend to read:

- Gastric bypass as described in the item 6.2 (Surgery plan);
- Hepatic biopsy during surgery: the extracted fragment will be forwarded to pathological analysis, as described in the item 6.1;
- Adipose tissue extraction during surgical procedure, as described in item 6.1.
- Medical examination of the subjects' overall clinical status;

7.4.2. Week 1 visit (surgical arm patients)

Summary of requested changes:

- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey (for both arms).

The protocol will amend to read:

- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey (for both arms).

7.4.3. Visit - Week 4, Month 3, Month 9, Month 15 and Month 21

Summary of requested changes:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;

The protocol will amend to read:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;

7.4.4. Visits – Month 6 and Month 18

Summary of requested changes:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Urine sample collection for DNA sequencing;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;

The protocol will amend to read:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Urine sample collection for DNA sequencing;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;

7.4.5. Month 12 and Month 24 Visits

Summary of requested changes:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose, for both arms;

The protocol will amend to read:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Urine sample collection for DNA sequencing;
- Assessment of capillary glucose, for both arms;

8. Procedures Flowchart

Summary of requested changes:

	Year 1							Year 2						
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Nutritional assessment	~~X~~	X			X	X	X	X	X	X	X	X	X	X
Upper digestive endoscopy (only for surgical arm)		X												
Urine samples for DNA sequencing		X						X		X		X		X
Hepatic biopsy			X											

* Applicable for patients randomized to the intervention group (surgical procedure), excluding Nutritional Assessment that will be made for both groups.

** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days based on randomization visit (baseline) for clinical arm patients and based on surgery date for intervention arm patients.

The protocol will amend to read:

	Year 1							Year 2						
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Nutritional assessment	~~X~~	X			X	X	X	X	X	X	X	X	X	X
Upper digestive endoscopy (only for surgical arm)		X												
Urine samples for DNA sequencing		X						X		X		X		X
Hepatic biopsy			X											

* Applicable for patients randomized to the intervention group (surgical procedure), excluding Nutritional Assessment that will be made for both groups.

** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days based on randomization visit (baseline) for clinical arm patients and based on surgery date for intervention arm patients.

Amendment 2

Title of Project:
PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

5.2. SECONDARY ENDPOINTS

Summary of requested changes:
1. Diabetic Retinopathy reversal

The protocol will amend to read:
1. Diabetic Retinopathy reversal

7.1. SELECTION/SCREENING

Summary of requested changes:
- ~~Eye assessment with retinoscopy, fundus photography and visual acuity test~~ Color retinography and angiofluoresceinography to evaluate the presence of diabetic retinopathy;

The protocol will amend to read:
Color retinography and angiofluoresceinography to evaluate the presence of diabetic retinopathy;

7.4.4. Visits – Month 6 and Month 18

Summary of requested changes:
- ~~Eye assessment with retinoscopy, fundus photography and visual acuity test~~ Color retinography and angiofluoresceinography to evaluate the presence of diabetic retinopathy;

The protocol will amend to read:
Color retinography and angiofluoresceinography to evaluate the presence of diabetic retinopathy;

7.4.5. Month 12 and Month 24 Visits

Summary of requested changes:
- ~~Eye assessment with retinoscopy, fundus photography and visual acuity test~~ Color retinography and angiofluoresceinography to evaluate the presence of diabetic retinopathy;

The protocol will amend to read:
Color retinography and angiofluoresceinography to evaluate the presence of diabetic retinopathy;

7.5. PROCEDURES FLOWCHART

Summary of requested changes:

	Year 1							Year 2						
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Retinography	~~X~~	X						X		X		X		X
~~Refraction test~~	~~X~~							~~X~~		~~X~~		~~X~~		~~X~~
~~Funduscopy~~	~~X~~							~~X~~		~~X~~		~~X~~		~~X~~
Angiofluoresceinography ~~(if necessary)~~	~~X~~	X						X		X		X		X

The protocol will amend to read:

	Year 1							Year 2						
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Retinography		X						X		X		X		X
Angiofluoresceinography		X						X		X		X		X

Amendment 1

Title of Project:
PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

Title page

Summary of requested changes:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
João Eduardo Salles Nunes
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Monica de Aguiar Medeiros

The protocol will amend to read:

Associated Researchers

Tarissa Beatrice Zanata Petry
Pedro Paulo Caravatto
Carlos Aurélio Schiavon
José Luís Correa
João Eduardo Salles Nunes
Cristina Mamédio Aboud
Mariangela Correa
Tiago Veiga Pereira
Debora Gitahy Reis
Monica de Aguiar Medeiros

7.1. SELECTION/SCREENING

Summary of requested changes:

- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, ~~eyes, ears, nose, throat,~~ heart, lungs, ~~breasts,~~ abdomen~~,~~ ~~external genitalia, limbs, muscles,~~ and neurological system;

The protocol will amend to read:

- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, heart, lungs, abdomen and neurological system;

7.1. SELECTION/SCREENING

Summary of requested changes:

- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.

The protocol will amend to read:

- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.

7.1. SELECTION/SCREENING

Summary of requested changes:

- Vital signs checking, including weight, height, heart rate, blood pressure, axillary temperature, and respiratory frequency;
~~- Subject randomization if all inclusion criteria and no one exclusion criteria were met;~~
~~- Dispensing of drugs and materials needed for the subject until next visit.~~

The protocol will amend to read:

- Vital signs checking, including weight, height, heart rate, blood pressure, axillary temperature, and respiratory frequency.

7.2. BASELINE VISIT

Summary of requested changes:

- ~~Give~~Provide information about surgical procedure for subjects randomized to the intervention group (surgery procedure);
- Guide patients to check blood glucose using a blood glucose meter that will be provided by the Health Research Unit. Blood glucose should be checked daily in the morning on an empty stomach and at night two hours after dinner. Patients who are considered glycemic controlled (fasting ≤ 150mg/dL and postprandial ≤180mg/dL) should measure blood glucose only 3 times a week;

- ~~Give~~Provide information about surgical procedure and drug treatment for subjects randomized to the intervention group (surgery procedure);
- ~~Give~~Provide information about drug treatment for subjects randomized to the control group (clinical treatment);

The protocol will amend to read:
- Provide information about surgical procedure for subjects randomized to the intervention group (surgery procedure);
- Guide patients to check blood glucose using a blood glucose meter that will be provided by the Health Research Unit. Blood glucose should be checked daily in the morning on an empty stomach and at night two hours after dinner. Patients who are considered glycemic controlled (fasting ≤ 150mg/dL and postprandial ≤180mg/dL) should measure blood glucose only 3 times a week;
- Provide information about surgical procedure and drug treatment for subjects randomized to the intervention group (surgery procedure);
- Provide information about drug treatment for subjects randomized to the control group (clinical treatment);

7.3. SURGERY

Summary of requested changes:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, ~~eyes, ears, nose, throat,~~ heart, lungs, ~~breasts,~~ abdomen~~, external genitalia, limbs, muscles,~~ and neurological system;

The protocol will amend to read:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, heart, lungs, abdomen and neurological system;

7.4.1. First to third postoperative day (surgical arm patients)

Summary of requested changes:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, ~~eyes, ears, nose, throat,~~ heart, lungs, ~~breasts,~~ abdomen~~, external genitalia, limbs, muscles,~~ and neurological system;

The protocol will amend to read:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, heart, lungs, abdomen and neurological system;

7.4.2. Week 1 Visit (surgical arm patients)

Summary of requested changes:
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose;
- Dispensing of drugs and materials needed for the subject until next visit;

The protocol will amend to read:
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose;
- Dispensing of drugs and materials needed for the subject until next visit;

7.4.3. Visits – Week 4, Month3, Month 9, Month 15 and Month 21

Summary of requested changes:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, ~~eyes, ears, nose, throat,~~ heart, lungs, ~~breasts,~~ abdomen~~,~~ ~~external genitalia, limbs, muscles,~~ and neurological system;

The protocol will amend to read:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, heart, lungs, abdomen and neurological system;

7.4.3. Visits – Week 4, Month3, Month 9, Month 15 and Month 21

Summary of requested changes:
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose, for both arms;
- Dispensing of materials and drugs needed until the next visit.

The protocol will amend to read:
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose, for both arms;
- Dispensing of materials and drugs needed until the next visit.

7.4.4. Visits – Month 6 and Month 18

Summary of requested changes:
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, ~~eyes, ears, nose, throat,~~ heart, lungs, ~~breasts,~~ abdomen~~,~~ ~~external genitalia, limbs, muscles,~~ and neurological system;

The protocol will amend to read:

- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, heart, lungs, abdomen and neurological system;

7.4.4. Visits – Month 6 and Month 18

Summary of requested changes:

- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose, for both arms;
- Dispensing of materials and drugs needed until the next visit.

The protocol will amend to read:

- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Assessment of capillary glucose, for both arms;
- Dispensing of materials and drugs needed until the next visit.

7.4.4. Month 12 and Month 18 Visits

Summary of requested changes:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Assessment of capillary glucose, for both arms;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.

The protocol will amend to read:

- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Assessment of capillary glucose, for both arms;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 30, 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.

8. Procedures Flowchart

Summary of requested changes:

	Year 1							Year 2						
Assessments	Screen**	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24

* Applicable for patients randomized to the intervention group (surgical procedure).

** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days based on randomization visit (baseline).

The protocol will amend to read:

	Year 1										Year 2			
Assessments	Screen**	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24

* Applicable for patients randomized to the intervention group (surgical procedure).

** The range established between the screening visit and the baseline will be +/- 30 days. The range established for the other visits will be of +/- 7 days based on randomization visit (baseline).

PROSPECTIVE, OPEN, RANDOMIZED, UNICENTER STUDY COMPARING ROUX-EN-Y GASTRIC BYPASS WITH THE BEST CLINICAL TREATMENT REGARDING IMPROVEMENT OF MICROVASCULAR COMPLICATIONS OF TYPE 2 DIABETES MELLITUS IN OBESE PATIENTS

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1. LIST OF ABBREVIATIONS AND DEFINITIONS OF TERMS

Abbreviation	Term
ADA	American Diabetes Association
AMP	Adenosine monophosphate
Anti-GAD	Antibody anti-glutamic acid decarboxylase
BMI	Body mass index
CDC	Centers for Disease Control and Prevention
CONEP	National Research Ethics Committee
DCCT	Diabetes Control and Complications Trial
DPP-IV	Dipeptidyl peptidase-IV
FDA	Food and Drug Administration
GIP	Gastric inhibitory polypeptide
GLP-1	Glucagon-like peptide-1
HbA1C	Glycosylated hemoglobin A1c
HDL	High density lipoprotein
HIV	Human immunodeficiency virus
IRB	Internal Review Board
ISO	International Organization for Standardization
Kg/m^2	Kilograms per square meter
LDL	Low density protein
MDRD	Modification of diet in renal disease
SF-36	Health-related and general quality of life survey (Short form-36)
T2DM	Type 2 diabetes mellitus
UKPDS	United Kingdom Prospective Study
USA	United States of America

2. INTRODUCTION

T2DM is a heterogeneous disorder characterized by defects in insulin secretion and sensitivity. Insulin resistance is the initial event in T2DM, and beta-cell function gradually declines until hyperglycemia is evident. Many mechanisms have been suggested as causes of insulin resistance, such as increase in non-esterified fatty acids, inflammatory cytokines and adipokines. In addition, endothelial and beta-cell dysfunction, glucotoxicity, lipotoxicity, defective incretin action (GLP-1 and GIP), and deposit of amyloid substance are also implicated in its physiopathology [STUMVOLL, 2005; NYENWE, 2011; DeFRONZO, 2009].

Pathogenesis of T2DM

Individuals prone to develop T2DM inherit certain genes involved in the development of insulin resistance, according to different tissues [DeFRONZO, 1988; ERIKSSON, 1989; PENDERGRASS, 2007; GROOP, 2008]. In the liver, one of the signs of insulin resistance is increased glucose production [DeFRONZO, 1989], whereas in the muscles, inhibited glucose uptake is evident, causing postprandial hyperglycemia [FERRANNINI, 1988]. In adipocytes, insulin resistance causes lipolysis and release of free fatty acids (lipotoxicity) [BAYS, 2004], with consequent deposition in the hepatocytes, myocytes, beta-cells and endothelium (accelerating atherosclerosis), which leads to increased production of inflammatory adipocytokines and worsening of insulin resistance [DeFRONZO, 2009].

Other mechanisms that may be involved in the pathogenesis of T2DM are hyperglucagonemia, produced by pancreatic alpha-cells and leading to enhanced hepatic glucose production (BARON, 1987], and increased renal glucose reabsorption [RAHMOUNE, 2005; DeFRONZO, 2009].

Although genetic mechanisms are involved in the development of insulin resistance, the T2DM epidemic is linked to the obesity epidemic [DeFRONZO, 1978] and sedentary lifestyle [KOIVISTO, 1986] of the Western population. Obesity and sedentary lifestyle, in combination with a genetic predisposition, lead to beta-cell overload, promoting a greater secretion of insulin in response to defective insulin action. Gradually, this overload results in beta-cell failure, which occurs when there is loss of approximately 80% of these cells' mass in the pancreas [WEYER, 2001; BUTLER, 2003]. Thus, these individuals develop fasting hyperglycemia, progressing to T2DM [DeFRONZO, 2009].

Incidence of T2DM and its Complications

The World Health Organization estimated that in 2000 there were approximately 170 million diabetics in the world. This figure is believed to double by 2030, reaching about 336 million individuals. In the Americas, the number of cases of type 2 diabetes will increase from 33 million to 66.8 million in the same period.

These patients' glycemic control is particularly difficult, as it has been shown in a study conducted in Brazil, in which 85% of patients had serum glycosylated hemoglobin (HbA1c) level > 7.0%, which is frequently associated with microvascular complications such

as nephropathy, retinopathy, and neuropathy [WILD, 2004; MENDES, 2010; REMUZZI, 2002; WATKINS, 2003; YOUNG, 1993].

In the United States, the number of people who started treatment for end-stage renal disease caused by diabetes increased from 2,600 in 1980 to 48,374 in 2008. In 2006, a survey conducted by the CDC found 66,000 nontraumatic lower limb amputations in diabetics in the United States. In addition, in 2009, 19.7% of U.S. diabetic adults had visual acuity impairment.

Clinical Treatment of T2DM

The United Kingdom Prospective Study (UKPDS) study showed that early intensive glycemic control reduces the risk of developing microvascular complications in T2DM patients in medium [UKPDS, 1998] and long terms [HOLMAN, 2008]. However, the Diabetes Control and Complications Trial (DCCT) reported a paradoxical worsening of microvascular complications, such as retinopathy and neuropathy, after faster glycemic control [DCCT, 1993]. The safety and efficacy of a rigorous glycemic control have been questioned in recent studies [PATEL, 2008; DUCKWORTH, 2009; GERSTEIN, 2008; BUCHWALD, 2009]. The concept of "metabolic memory" together with side effects of an intensive pharmacological treatment may explain the discrepancy of these studies' results.

Despite the development of new medications for T2DM, these drugs are equally or less effective than the three previous classes of drugs (insulin, biguanide, and sulfonylurea) to reduce blood glucose, as shown in the table below (Table 1). Furthermore, these new drugs are more expensive [NATHAN, 2007a]. In addition, with the evolution of the disease and because it is a multicausal disease, combinations of various classes of antidiabetic medications are necessary to achieve disease control. And most of the time such control is not achieved, even with so many pharmacological options [DeFRONZO, 2009; MENDES, 2010].

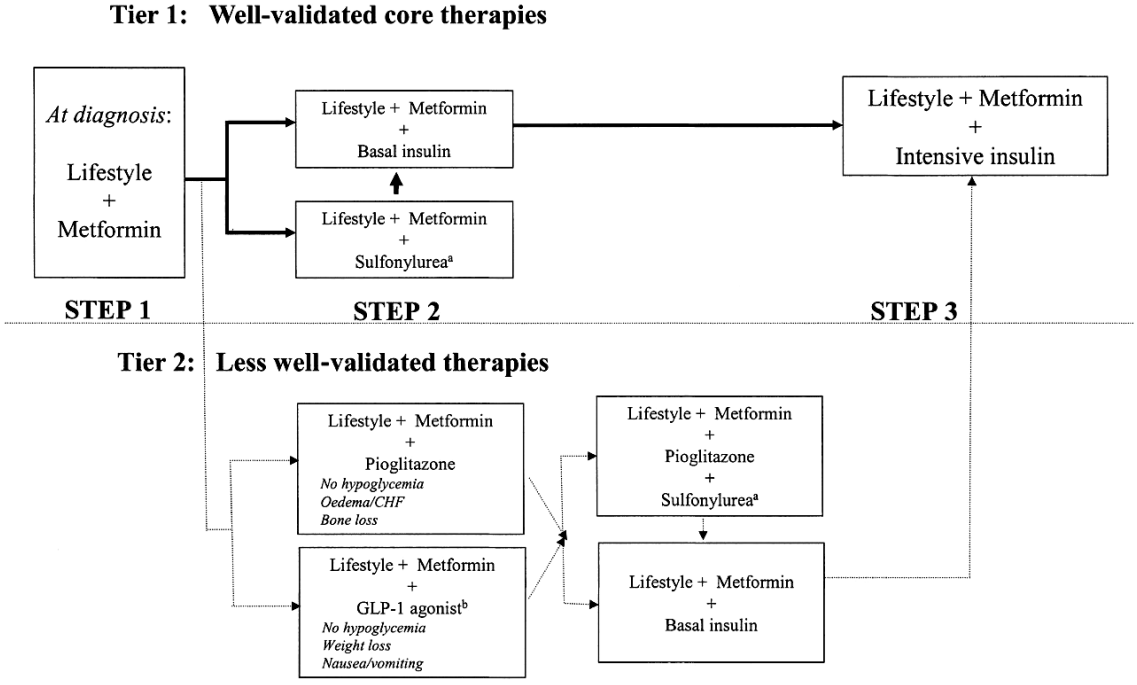
Nine classes of medications for T2DM have been approved so far by the FDA in the United States, as shown in the table bellow:

Antidiabetic Drugs Approved in the United States			
Drug	Route of Administration	Market Release Date	% of reduction in HbA1c when used as a monotherapy
Insulin	Parenteral	1921	≥2.5
Sulfonylurea	Oral	1946	1.5
Biguanide	Oral	1957	
Metformin	Oral	1995	1.5
Alpha-glucosidase inhibitors	Oral	1995	0.5-0.8
Thiazolidinedione	Oral		0.8-1.0
Pioglitazone	Oral	1999	
Glinide	Oral	1997	1.0-1.5
GLP analogs	Parenteral	2005	0.6
Amylin analogs	Parenteral	2005	0.6
DPP-IV inhibitors	Oral	2006	0.5-0.9

Adapted from NATHAN, 2007.

In 2009, a consensus algorithm for the initiation and adjustment of therapy was published in Diabetes Care, as shown below.

Fig. 1. Consensus algorithm for the initiation and adjustment of therapy in Diabetic Patients'



Despite these recommendations, DeFronzo suggests that the best clinical treatment for T2DM is a combined therapy acting against the causes of the disease and preserving

the maximum mass of pancreatic beta-cells. In the liver, both metformin [CUSI, 1996] and thiazolidinedione [BAYS, 2004] are potent insulin sensitizers and inhibit the T2DM-typical increased gluconeogenesis. In the muscles, thiazolidinedione has the same insulin sensitization action [MIYAZAKI, 2001]. Whereas thiazolidinedione acts on the insulin signaling pathway, metformin acts on AMP kinase pathway, both leading to an additive effect in reducing HbA1c without causing hypoglycemia, since they do not affect insulin secretion. In the adipose tissue, thiazolidinedione has also an excellent insulin sensitization action, leading to inhibition of lipolysis, removing fat for from the muscles, liver, and beta-cells, improving lipotoxicity, enhancing and preserving beta-cell function [BAYS, 2004; DeFRONZO, 2009]. Although leading to weight gain and increased chance of hypoglycemia, insulin initiation is necessary if oral medications were not sufficient to achieve HbA1c target levels.

There is evidence that GLP-1 analogs can also preserve beta-cell function [KLONOFF, 2008]. In addition, these analogs reduce hepatic glucose production in the liver, decrease glucagon secretion by pancreatic alpha-cells, reduce gastric emptying in the gastrointestinal tract by and resolve GLP-1 deficit, while reducing appetite and leading to weight loss. It is worth mentioning that the GLP-1 action of increasing insulin secretion dissipates when normoglycemia is achieved, preventing hypoglycemia. There is not enough evidence that inhibitors of DPP-IV, an enzyme that degrades incretins (such as GLP-1), are able to preserve beta-cells. However, these inhibitors also act by reducing hepatic glucose production, while stimulating insulin secretion and decreasing glucagon secretion [DeFRONZO, 2009].

Obesity and T2DM

Worldwide, obesity prevalence more than doubled between 1980 and 2008. In 2008, 10% of men and 14% of women in the world were obese (BMI ≥ 30.0 Kg/m^2) and 35% of adults older than 20 years were overweight (BMI ≥ 25.0 and < 30 Kg/m^2).

Recent studies [ECKEL, 2011] have found associations between T2DM and obesity involving pro-inflammatory cytokines (tumor necrosis factor, interleukin-6), insulin resistance, changes in fatty acid metabolism, and cellular processes such as mitochondrial dysfunction and endoplasmic reticulum stress. These interactions are complex and their importance has not been clearly defined.

Nevertheless, there is no doubt that obesity plays a major role in the pathogenesis of diabetes. This relationship can be observed when analyzing data from epidemiological studies.

In the U.S. population, between the ages of 20 and 74 years there was a significant increase in the prevalence of diabetes between 1976–1980 and 1999–2000 (3.3% to 5.8%). However, these trends varied according to the BMI level. In individuals with BMI ≥35 kg/m^2, diabetes increased considerably (from 4.9% in 1960, to 8.6% during 1976–1980, to 15.1% in 1999–2000). [GREGG, 2004]

Therefore, the proportion of diagnosed diabetes cases increased from 41 to 83% among individuals with BMI ≥35 kg/m^2, while changes in diabetes prevalence in subjects

with lower BMI's were modest, leading to imperceptible changes in the total number of diagnosed cases. [GREGG, 2004]

There is evidence that the common link between obesity and diabetes is the proinflammatory state usually seen in patients with metabolic syndrome.

The metabolic syndrome is defined as a conjunction of metabolic risk factors, as follows [HUMPHREY, 1998; SCOTT, 2004]

- atherogenic dyslipidemia with serum elevation of triglycerides, apolipoprotein B and small low-density lipoprotein (LDL) associated with low high-density lipoprotein (HDL) cholesterol;
- elevated blood pressure;
- elevated glycemia and insulin resistance;
- proinflammatory state;
- prothrombotic state.

The National Cholesterol Education Program Adult Treatment Panel III report proposed the following criteria for diagnosis of metabolic syndrome (3 of the 5 following criteria must be met for its diagnosis) [NCEP, 2002]

- increased waist circumference (≥ 102 cm in men and ≥ 88 cm in women);
- elevated tryglicerides (≥ 150 mg/dL);
- reduced HDL cholesterol (< 40 mg/dL in men and < 50 mg/dL in women);
- elevated blood pressure (≥ 130x85 mmHg or on treatment for hypertension);
- elevated glucose (≥ 100 mg/dL).

Surgical Treatment of T2DM

Gastric bypass and other types of metabolic surgery have shown to be effective in the treatment of T2DM, reducing the mortality rate in the long term when compared with optimal clinical treatment [BUCHWALD, 2009; SJOSTROM, 2004, 2007] .The term "bariatric" is gradually being replaced by "metabolic", since the surgery previously recommended for the treatment of morbidly obese individuals (defined by BMI greater than 40 kg/m^2 or greater than 35 kg/m^2 associated with comorbidities that are difficult to control) has demonstrated excellent results in terms of diabetes remission, even in patients with BMI lower than 35 kg/m2. Thus, the use of BMI as the single criterion for indication for surgical treatment has been questioned by several medical associations, especially for diabetic patients with BMI < 35 kg/m2 and whose disease is difficult to control using pharmacological treatment.

Therefore, a "metabolic" surgery is currently defined as any surgical procedure in which there is any anatomical alteration in the gastrointestinal tract by means of a diversion of food passage, resulting in improved metabolic control in patients with T2DM. Such result does not depend on weight loss and, in some cases, it can be observed some days or weeks after the surgical procedure, long before considerable weight loss. A growing number of these surgeries are being performed every year worldwide, and they are expected to be part of the algorithms for diabetes therapy, combined with changes in lifestyle and drug therapy.

Several studies have shown that the best results regarding T2DM remission are obtained after procedures that include proximal bowel diversion, performed in the Roux-en-Y gastric bypass or biliopancreatic diversion. In a 14-year follow-up, T2DM remission was found in 83% of the patients who underwent gastric bypass. Similar data were found in 240 patients undergoing the same type of surgery, with 83% remission in a mean follow-up of 10 years [BUCHWALD, 2004]. Biliopancreatic diversion, in turn, offers superior results regarding T2DM remission, found in 97% of patients with a mean follow-up of 10 years. However, there is a greater number of long-term complications, especially nutritional complications (15 to 20% of malnutrition in the long term) [BUCHWALD, 2004; PORIES, 2008]. In this context, Roux-en-Y gastric bypass carries more advantages because it is a more standardized surgery, with longer follow-up studies, showing a low mortality rate (0.30%), with few major complications in the long term (0.7%) [BUCHWALD 2004]. Restrictive procedures, such as adjustable gastric band, have lower rates of T2DM remission (45%). Such results are dependent on caloric restriction and weight loss and are not maintained in the long term, when is weight recovery is common.

Studies have shown that the mechanisms responsible for metabolic improvement after a Roux-en-Y gastric bypass include weight loss, decreased insulin resistance, increased insulin production, proximal bowel diversion, increased incretins levels, and bile flow alteration [LeROUX, 2006; BORG, 2006; LeROUX, 2007; THALER, 2009; POURNARAS, 2010]. In relation to renal function, other studies also found increased urine production and sodium excretion after gastric bypass in rats [BUETER, 2011], as well as improvement of urinary and systemic inflammatory markers in patients [BUETER, 2010]. A retrospective study involving 83 T2DM patients with previous nephropathy and/or retinopathy showed that, after gastric bypass surgery, nephropathy and retinopathy remission were very common, with an 85.2% reduction in the albumin/creatinine ratio and improvement in retinal images in 19% of cases (manuscript in preparation).

In recent decades, T2DM therapy has not been exclusively aimed at controlling glycemic levels but is instead focused on preventing macrovascular and microvascular complications (ISMAIL-BEIGI, 2011].

Every new T2DM treatment must be safe and effective, not only to correct hyperglycemia, but also to prevent or mitigate the complications of this chronic disease. Only one prospective study showed that biliopancreatic diversion can reverse diabetic nephropathy [IACONELLI, 2011]. This surgery is not commonly performed worldwide because of the nutritional complications in the long term. The only randomized controlled trial conducted in patients with T2DM undergoing metabolic surgery focused only on glycemic control. Furthermore, in this study, patients underwent placement of adjustable gastric band [DIXON, 2008], showing improvement in glycemic control directly related to weight loss with inferior results in comparison with other surgical techniques.

Roux-en-Y gastric bypass is considered the gold standard for bariatric surgery, and it is the type of surgery most often performed in the world to control obesity [SCHAUER, 2003]. It consists of gastric volume reduction and exclusion of duodenum and proximal jejunum from food transit. As it has been found in several studies [PORIES, 1995; RUBINO, 2002; RUBINO, 2004], this type of operation promotes reduction of glucose levels in diabetic

patients a few days after the procedure, even before there is an effective weight loss. Based on these findings, it was possible to suggest that the T2DM control mechanism achieved by this procedure occurs mainly as a result from the alteration in the normal gastrointestinal transit, instead of resulting exclusively from weight loss. Recently this group of researchers [Cohen, 2012] published the long-term results of surgical treatment for T2DM in patients with BMI between 30-35 kg/m^2. After 6 years of follow-up, has been shown that 88% of 66 patients operated got on remission, i.e. without antidiabetic and HbA1c less than 6.5% and control of dyslipidemia and hypertension.

Some hypotheses have been proposed to explain the effect of this surgery on T2DM. The most accepted hypothesis is related to the influence of the duodenum and proximal jejunum (proximal bowel mechanism) as key factors in the control of insulin resistance in T2DM patients. Rubino (2004), in an experimental study with a model of duodenal exclusion in nonobese diabetic Goto Kakizaki rats, demonstrated impressive improvement in glycemic levels of these animals compared with the control group. In another study (RUBINO 2006), the same author concluded that duodenal-jejunal exclusion was more important than ileal stimulation (distal bowel mechanism) for the normalization of glucose levels in these animals. Rubino suggested that a hormone secreted by the duodenum has an "anti-incretinic" effect and with the exclusion of this intestinal segment, this blockade would disappear and the opposite effect would dominate, i.e., reduced insulin resistance and increased insulin secretion.

Another hypothesis postulates that the rapid arrival of nutrients in the ileum (distal intestine mechanism) strengthens the effect of GLP-1, which is an incretin hormone secreted by ileal L cells in response to the arrival of undigested food. This hormone would stimulate insulin secretion and have an antiapoptotic effect on pancreatic beta-cells, as mentioned above, leading to glycemic control and preservation of pancreatic endocrine cells [MOO & RUBINO, 2008; LEE, 2008; RUBINO, 2006; RUBINO, 2002]. Roux-en-Y gastric bypass produces the two mechanisms explained above.

These studies also showed that the hypothesis of weight loss especially resulting from lower intake of carbohydrates and lipids has been demystified, since D-xylose and fecal fat tests showed no significant difference when compared with controls [RUBINO, 2006].

However, it seems that there is a change in the metabolism of free fatty acids, which, in high concentrations, lead to insulin resistance. In addition, after the surgical procedure, reduction of free fatty acids may have an influence on the improvement of insulin resistance [SNOW, 1993; BOURDAGES, 1994].

Gastrointestinal anatomy rearrangement may also be related to the control of T2DM based on the effect of other hormones. Some of these hormones are: peptide YY (PYY) and GIP, which are secreted in the proximal and distal bowel and are also related to the beta-pancreatic functioning and satiety; as well as ghrelin, which acts as an orexigenic signal and it is secreted in gastric fundus and duodenum. Besides that, ghrelin has direct and counter regulatory diabetogenic effects [DE PAULA, 2006; COHEN, 2006 e 2012].

These assumptions were also tested in studies involving humans with BMI < 35 Kg/m² who underwent duodenal exclusion [COHEN, 2007] and ileal interposition [DE PAULA, 2006]. The initial results from these studies seem promising and suggest a new possibility

of T2DM control in patients with overweight and grade 1 obesity. What is really interesting regarding these satisfactory results is that there is evidence of improvement in the beta-cell function of diabetic patients who underwent surgery when compared with a group of patients with normal glucose tolerance, and that glycemic control was mainly caused by a direct antidiabetic unrelated to the patients' weight change [KLEIN & COHEN, 2012].

3. RATIONALE

Surgery in patients with low BMI

Excellent results observed after gastric bypass in patients with diabetes and BMI> 35 kg/m^2 along with evidence of weight-independent mechanisms antidiabetic led to considering surgery to less obese diabetics. Clinical practice of bariatric surgery, however, is based on a 1991 NIH consensus, which limits the use of bariatric surgery in patients with BMI ≥ 35 kg / m2 with comorbidities, such as diabetes.

Patients with BMI between 30 and 35 kg/m2 (obesity grade I) are most diabetic patients. Millions of these suffer from diabetes uncontrolled despite lifestyle changes and pharmacotherapy; and does not enter the NIH criteria for bariatric surgery. Therefore, we propose to evaluate the effect and safety of this surgery for type 2 diabetic patients with obesity grade I.

Surgery x Clinical treatment

The medical community is confronted with many different studies using various methodologies to investigate the best pharmacological treatment for T2DM. The treatment algorithm offers several different options according to the stage of the disease (which is different in each study). In addition, new drugs are being developed over the years, but are not always a guarantee of effective T2DM control [MENDES, 2010]. Furthermore, these drugs do not prevent the development of this disease, consequently increasing the risks of microvascular and macrovascular complications.

Conversely, there is considerable evidence that surgery can be an adequate tool to promote T2DM remission in patients who are unresponsive to clinical treatment. Gastric bypass surgery is one of the most popular metabolic surgeries in the world, but its effects on microvascular and macrovascular complications of T2DM have not been established. Specialists suggest that the rapid and uncontrollable decrease in blood glucose adds to the concern that the surgery may paradoxically cause exacerbation of microvascular complications [LEOW, 2005], whereas gradual improvement in blood glucose before gastric bypass surgery may prevent this paradoxical worsening, leading to an interruption of this process, or even retinopathy, nephropathy, and neuropathy remission.

However, there are no studies comparing the results of these two types of treatment (clinical vs. surgical) in a similar population and assessing the development of microvascular complications of T2DM. Therefore, in order to clarify such doubts, it is necessary and extremely desirable to conduct a randomized controlled trial comparing gastric bypass with

the best and most modern clinical treatment. Its findings could have a direct impact on hundreds of millions of diabetics by allowing the inclusion of surgical treatment as a safe and feasible therapeutic option for a significant portion of these patients.

4. STUDY AIM

The aim of this prospective, open, randomized study is to evaluate the effects of Roux-en-Y gastric bypass in the control of diabetic nephropathy in diabetic patients with BMI between 30 and 35 kg/m^2.

5. ENDPOINTS

5.1. PRIMARY ENDPOINT

The primary endpoint will be the proportion of patients that present normalization of the albumin/creatinine ratio in isolated urine samples (normal value considered as an albumin/creatinine ratio of less than 30 µg/mg).

5.2. SECONDARY ENDPOINTS

1. Retinopathy reversal
2. Development or worsening of peripheral neuropathy
3. Discontinuation of pharmacological therapy for T2DM
4. Glycemic control (fasting glucose level < 100 and HbA1c < 6.5%)
5. Normalization of blood pressure (systolic blood pressure < 130 mmHg and diastolic blood pressure < 80 mmHg) (ADA, 2012]
6. Normalization of lipids (LDL < 100 mg/dL and < 70 mg/dL in patients with cardiovascular disease; HDL > 50 mg/dL, triglycerides < 150 mg/dL) (ADA, 2012]
7. Improvement on Quality of life (SF-36)

6. STUDY DESIGN AND STATISTICAL ANALYSIS

6.1. STUDY DESIGN

This is a prospective, open, randomized study involving 72 patients with microvascular complications of T2DM and obesity, who will undergo gastric bypass (Roux-en-Y gastric bypass) or receive best medical treatment.

Thirty-six obese patients (BMI between 30 and 35 kg/m^2) with microvascular complications of T2DM will undergo gastric bypass. Those patients with HbA1c ≥ 10.0%

who were randomly allocated to the surgical arm will receive pharmacological treatment with the purpose of improving glycemia (HbA1c ≤10%) for up to 3 months, which has proven to improve outcomes in the postoperative period [DIABETES, 2011]. Those who did not reach this goal will be exclude from the study. Particular attention will be given to avoid hypoglycemia, through capillary glycemia control, especially in those patients taking insulin. Other comorbidities, such as hypertension and dyslipidemia, will be compensated according to the latest recommendations of the American Diabetes Association [ADA, 2012]. The surgical procedure will consist of a laparoscopic surgery performed by an experienced surgeon (approximately 4,500 bariatric surgeries), who is accredited with Oswaldo Cruz German Hospital as surgeon of excellence by the SBCBM/SRC program since 2009 and is the current president of the Brazilian Society of Bariatric and Metabolic Surgery).

Regarding medication to be used, if there is no contraindication, metformin will be maintained in the postoperative period while fasting glycemia is above 100 mg/dL. Anti-antihypertensive drugs and medications for dyslipidemia will be maintained in the postoperative period, unless there are any contraindications. A supplement of micronutrients (vitamins and mineral salts) will be prescribed to all patients who undergo surgery. Patients allocated to the control group will receive the same supplementation if necessary.

The control group will include thirty-six patients with microvascular complications of T2DM who will receive the best clinical treatment available, which will consist of metformin, glitazones, incretin therapy (DPP4 inhibitor and GLP-1 analogs) and insulin, if necessary [DeFRONZO, 2009]. In addition, other comorbidities, such as hypertension and dyslipidemia, will be compensated according to ADA recommendations [ADA, 2012].

6.2. SURGICAL PROPOSAL

1) Pneumoperitoneum closed with Veress needle
2) Identification of Treitz angle
3) Measurement of biliary loop (100 cm)
4) Bowel transection with linear stapler (white load)
5) Measurement of small intestine (150 cm)
6) Laterolateral Entero-anastomoses (white load)
7) Construction of gastric pouch distant about 3 cm from the esophageal-gastric junction with stomach section in the small curvature.
8) Linear cutting anastomosis (gastrojejunostomy) from about 1 to 1.2 cm
9) Anastomosis integrity evaluation by methylene blue test and/or perioperative air.

Expected surgical time: 60 minutes

6.3. INCLUSION CRITERIA

- Male and female adult patients with microalbuminuria (more than 30 mg and less than 300 mg or more of urinary albumin per 24hours), with or without other microvascular complications of T2DM, receiving pharmacological treatment for the disease, which may or may not include the use of insulin;
- Age between 18-65 years;
- BMI between 30 and 35 Kg/m^2;
- 15-year or less after T2DM diagnosis;
- Negative anti-GAD;
- Fasting C-peptide higher than 1 ng/ml, increasing in the postprandial period (two hours after mixed meal, ENSURE plus approximately 500 Kcal).

6.4. EXCLUSION CRITERIA

- Patient's refusal to participate;
- Autoimmune DM;
- Previous abdominal surgeries that may make surgery more difficult, increasing the surgical risk;
- Previous malabsorptive and restrictive surgeries;
- Pregnant women and nursing mothers;
- Recent history of neoplasia (< 5 years), except for non-melanoma skin neoplasms
- History of liver disease – liver cirrhosis –, active chronic hepatitis, active hepatitis B and hepatitis C;
- Malabsorptive syndromes and inflammatory bowel disease;
- Cardiovascular event (acute myocardial infarction, acute coronary syndrome, angioplasty, or bypass in the last 6 months);
- Angina;
- Pulmonary embolism or severe thrombophlebitis in the last 2 years;
- Positive HIV serum testing;
- Psychiatric disorders, including dementia, active psychosis, severe depression, history of suicide attempts, use of illicit drugs, and excessive alcohol consumption in the last 12 months;
- Uncontrolled coagulopathy;
- Patients with severe retinopathy, nephropathy, and neuropathy (defined as high risk/advanced proliferative retinopathy or amaurosis; stage 5 of chronic kidney disease defined by glomerular filtration rate, patients who need dialysis or renal transplantation; stage 3 of peripheral neuropathy);
- Patients who participated in other clinical trials in the past 30 days.

6.5. RECRUITMENT & FOLLOW-UP

The recruitment period will last for 6 months from the beginning of the study (initiation visit), and follow-up will proceed for 24 months after participant inclusion in the study.

6.6. STATISTICAL ANALYSIS AND SAMPLE SIZE CALCULATION

6.6.1. STATISTICAL ANALYSIS

All analyzes will be performed according to the intention-to-treat principle. Missing data will be entered using previous approaches if appropriate [PEDUZZI 2002].

Data will be expressed as mean ± standard deviation (SD), median (interquartile range), or absolute number (or percentage), when appropriate.

For continuous data, independent groups will be compared by an unpaired t test for approximately normally distributed variables (with Welch's correction when deemed necessary). Within-group (pre- vs. post-) differences will be investigated by the t test for paired samples. Binary outcomes will be assessed by logistic regression models and/or Fisher's exact test. Logistic models will be fit using the backward stepwise selection procedure (cutoff ≤ 0.10). A similar approach will be applied for continuous dependent variables using multiple linear regression models. All data analyses will be performed using the Stata package (version 11.0, Stata Corp., College Station, TX, USA). Statistical significance level will be set at 5% (two-tailed).

6.6.2. SAMPLE SIZE CALCULATION

Previous estimates for the proportion of participants who are likely to achieve the primary endpoint (that is, a normal albumin/creatinine ratio) in the group allocated to clinical treatment (Pc) and in the group of surgical intervention (Ps) were obtained in the medical literature (BOUSSAGEON, 2011) and unpublished data of the group.

We determined the sample size required to detect an association with a 5% level of significance (α) assuming a randomized controlled trial with two independent groups.

This approach assumes that the null hypothesis (that $\Delta = 0$, where $\Delta = Ps - Pc$) will be accepted/rejected on the basis of a 2-sample test for equality of proportions with continuity correction data.

The statistical power was first calculated analytically in accordance with the method described by ZAR (1999). Analytically derived estimates of the power of the study were corroborated with an empirical approach, based on the Monte Carlo simulation. With this purpose, individual studies were simulated according to the methodology proposed by SUTTON et al. (2007) and data were analyzed using Fisher's exact test when the asymptotic assumptions were not valid (for example, counts lower than 5 in the 2x2 table). In the remaining cases, we used the Z test, where $Z = \theta/IF(\theta)$ and θ and $IF(\theta)$ are, respectively, the estimated logarithm of odds ratio and its asymptotic standard error. The empirical power was calculated as the proportion of 10,000 simulations that produced a two-tailed p value <

0.05. All analyzes were performed using the statistical package Stata 11.0 (Stata Corporation, College Station, TX, USA).

Thus, considering the data in the literature and unpublished data of the group, as well as a rate of loss to follow-up of 15% for both groups and a two-tailed type I error (α) of 5%, the final scenario is:

Statistical power of 80%

Scenario 1
Pc = 10% (probability of achieving the primary endpoint in the clinical group)
Pc = 50% (probability of achieving the primary endpoint in the surgical group)
Number of research subjects in each arm = 29
Total number of patients in the study = 58

Scenario 2
Pc = 5% (probability of achieving the primary endpoint in the clinical group)
Pc = 60% (probability of achieving the primary endpoint in the surgical group)
Number of research subjects in each arm = 16
Total number of patients in the study = 32

Scenario 3
Pc = 5% (probability of achieving the primary endpoint in the clinical group)
Pc = 70% (probability of achieving the primary endpoint in the surgical group)
Number of research subjects in each arm = 13
Total number of patients in the study = 26

The three previous scenarios are less conservative (considering Scenario 1 as the most conservative), with less chance of detecting a biological and statistically significant effect, worse with greater differences between Ps and Pc.

Statistical power of 90%

Scenario 4
Pc = 10% (probability of achieving the primary endpoint in the clinical group)
Pc = 50% (probability of achieving the primary endpoint in the surgical group)
Number of research subjects in each arm = 36
Total number of patients in the study = 72

Scenario 5
Pc = 5% (probability of achieving the primary endpoint in the clinical group)
Pc = 60% (probability of achieving the primary endpoint in the surgical group)
Number of research subjects in each arm = 20
Total number of patients in the study = 40

Scenario 6
Pc = 5% (probability of achieving the primary endpoint in the clinical group)
Pc = 70% (probability of achieving the primary endpoint in the surgical group)
Number of research subjects in each arm = 15
Total number of patients in the study = 30

These are more conservative scenarios (4, 5 and 6), com increased chance of detecting significant differences, worse with greater differences between Ps and Pc, as like in the previous scenarios.

As the best option to answer the question of this protocol, and assuming a conservative profile as a guarantee to obtain reliable results, we chose the conditions Ps and 50% PC 10%, with a statistical power of 90% (scenario 4). These parameters show an optimal number of research subjects in each arm of 36 patients, reaching a total of 72 patients in the study.

6.6.3. RANDOMIZATION AND ALLOCATION OF PATIENTS

Eligible patients will be randomly assigned with concealed allocation to either bariatric surgery or diet/lifestyle intervention. A biostatistician not otherwise associated with the study will be responsible for generating the computerized randomization schedule. An independent research assistant will manage and schedule the communication between participants and physicians.

Randomization process will be accomplished by the computer-generated permuted using block approach. Specifically, random blocks with predetermined sizes of at least two patients, at the latest four patients will be generated.

Concealed allocation will be ensured through the use of opaque and sealed envelopes (SNOSE approach), numbered sequentially. The envelope will be opened by the physician/investigator in the first patient assessment, where allocation order is irreversible.

6.7. STUDY FLOW CHART

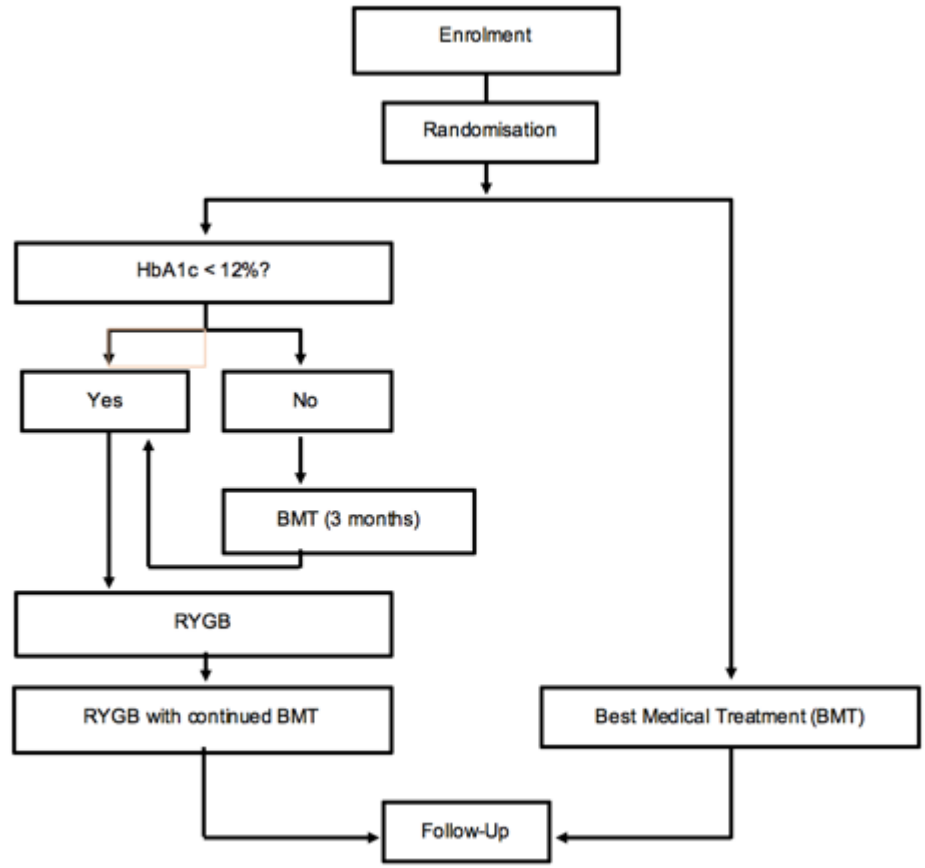


Figure 1. Simplified MOMS algorithm

6.8. CRITERIA FOR PATIENTS' WITHDRAWAL FROM THE STUDY

If, at any time, significant problems with the surgery are suspected, a re-operation may be performed. Should participants require withdrawal for any reason, follow-up phone calls will continue as scheduled. If a patient cannot be reached, then friends or relatives of the patient will be contacted,

Patients may withdraw of the study if:

- voluntary decision to withdraw;
- study is terminated.

7. STUDY PROCEDURES

Patients will undergo procedures according to the time intervals described in the Procedures Flow Chart (item 7.5).

7.1. SELECTION/SCREENING

The screening period will begin after the subjects voluntarily sign the informed consent form. Those subjects who provide consent will undergo the following screening procedures to determine their eligibility for the study:

- Medical anamnesis, which will include collection of information for the assessment of the subjects' clinical history, collection of demographic data (date of birth, sex, race, etc);
- Evaluation of inclusion and exclusion criteria;
- Medical examination of the subjects' overall clinical status;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, eyes, ears, nose, throat, heart, lungs, breasts, abdomen, external genitalia, limbs, muscles, and neurological system;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start date;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey.
- Quality of life assessment using the SF-36;
- Laboratory tests as described in the Flowchart of the Study and in Assessments and Tests;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement.
- Chest x-rays, thoracic, anteroposterior and lateral projections;
- Full abdominal ultrasound, under fasting and bladder repletion;
- Standard digital 12-lead electrocardiogram, including heart rate, rhythm, and RR, PR, QRS and QT intervals;
- Color Doppler echocardiogram. If significant changes are found, the physician in charge might decide to refer the subject to dypiramidol myocardial perfusion scintigraphy to better establish the patient's clinical picture;
- Upper digestive endoscopy, including H. pylori infection investigation and biopsy only if necessary;
- Eye assessment with retinoscopy, fundus photography and visual acuity test to evaluate the presence of diabetic retinopathy;
- Vital signs checking, including weight, height, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Subject randomization if all inclusion criteria and no one exclusion criteria were met;

- Dispensing of drugs and materials needed for the subject until next visit.

7.2. BASELINE VISIT

-Evaluation of all laboratory tests and imaging results;
- If the subject is found elegible for the study (meet all inclusion criteria and no exclusion criteria), the subject will be randomized for the study. The randomization procedure to be performed is described in Section 6.6.3;
- Subjects randomly assigned to the intervention group (surgery) who present HbA1c ≥ 10,0% will be clinically treated during 3 (three) months and will be reevaluated in a new baseline visit. If during this new baseline the subject still presents HbA1c ≥ 12,0%, he will be excluded from the study.
- At reevaluation, subjects should redo the following exams, procedures or assessments: medical evaluation, physical exam, nutritional assessment, serum pregnancy test if applicable, blood count, AST/ALT, sodium, potassium, urea, creatinine, coagulation, lipid profile, fasting glucose, HbA1c, urine type I, microalbuminuria, urinary creatinine, electrocardiogram, chest X-ray, vital signs measurement, weight and height;
- Dispensing of drugs and materials needed for the subject until next visit;
- Give information about surgical procedure and drug treatment for subjects randomized to the intervention group;
- Give information about drug treatment for subjects randomized to the control group.

7.3. SURGERY

- Up to 3-day hospitalization for surgical procedure. Those subjects randomized to the surgical arm of the study will be admitted to individual rooms and will receive full care during the period of hospitalization;
- Gastric bypass as described in the item 6.2 (Surgery plan);
- Medical examination of the subjects' overall clinical status;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, eyes, ears, nose, throat, heart, lungs, breasts, abdomen, external genitalia, limbs, muscles, and neurological system;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Vital signs checking, including weight, height, heart rate, blood pressure, axillary temperature, and respiratory frequency.

7.4. CLINICAL FOLLOW-UP

7.4.1. First to third postoperative day (surgical arm patients)

- Medical examination of the subjects' overall clinical status;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, eyes, ears, nose, throat, heart, lungs, breasts, abdomen, external genitalia, limbs, muscles, and neurological system;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of drugs and materials needed for the subject until next visit;

7.4.2. Week 1 Visit (surgical arm patients)

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey.
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of drugs and materials needed for the subject until next visit;

7.4.3. Visits - Week 4, Month 3, Month 9, Month 15 and Month 21

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, eyes, ears, nose, throat, heart, lungs, breasts, abdomen, external genitalia, limbs, muscles, and neurological system;

- Assessment and review of any adverse event occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of drugs and materials needed for the subject until next visit;

7.4.4. Visits - Month 6 and Month 18

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;
- Physical examination including any clinically significant changes in any of the systems: general appearance, skin, head, eyes, ears, nose, throat, heart, lungs, breasts, abdomen, external genitalia, limbs, muscles, and neurological system;
- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Eye assessment with retinoscopy, fundus photography and visual acuity test to evaluate the presence of diabetic retinopathy;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests section;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of drugs and materials needed for the subject until next visit;

7.4.5. Month 12 and Month 24 Visits

- Medical examination of the subjects' overall clinical status;
- Record of all medications concomitantly used by the subjects. This record will include: drug trade name, pharmacological name, dosage, route of administration, frequency of use, therapeutic indication, and start and end date of drug use. The reasons for changing or discontinuing concomitant medications must be explained;

- Assessment and review of any adverse events occurred after signing the informed consent. The adverse events will be classified according to the CTCAE, version 3.0;
- Nerve conduction studies for initial assessment of sensory and motor functioning of the peripheral nervous system;
- Nutritional assessment containing anthropometric evaluation (weight and height), clinical evaluation, and nutritional survey;
- Quality of life assessment using the SF-36;
- Eye assessment with retinoscopy, fundus photography and visual acuity test to evaluate the presence of diabetic retinopathy;
- Laboratory tests as described in the Flowchart of the Study and in the Assessments and Tests sections;
- Meal tolerance test. This test will consist of the measurement of fasting glucose, insulin, and C peptide. Immediately after collection, patients will take a nutritional supplement containing 500cal (Ensure FOS). New collections (glucose, insulin, and C peptide) will be performed 60, 90, 120, 150, and 180 minutes after the nutritional supplement intake. During the test, patient should remain at rest and they will only be allowed to consume the supplement;
- Vital signs checking, including weight, heart rate, blood pressure, axillary temperature, and respiratory frequency;
- Dispensing of drugs and materials needed for the subject until next visit.

8. Procedures Flowchart

	Year 1							Year 2						
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Informed consent	X													
Anamnesis	X													
Inclusion/exclusion criteria	X	X												
Medical examination	X		X	X	X	X	X	X	X	X	X	X	X	X
Randomization		X												
Concomitant medications	X	X		X	X	X	X	X	X	X	X	X	X	X
Physical examination	X							X		X		X		X
Adverse events	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Nerve conduction studies	X							X		X		X		X
Nutritional assessment	X				X			X		X		X		X
SF-36 administration	X									X				X
Serum pregnancy test	X													
Complete blood count	X													
AST/ALT	X													
Sodium and potassium	X													
Urea and creatinine	X						X	X	X	X	X	X	X	X
Coagulogram (PT and APTT)	X													
Lipid profile (total cholesterol, LDL, HDL and tryglicerides)	X							X		X		X		X
Fasting glycemia				X	X	X	X		X		X		X	
HbA1c	X						X	X	X	X	X	X	X	X
Microalbuminuria (isolated sample)	X									X				X
Urinary creatinine	X									X				X
Iron and ferritin	X							X		X		X		X
HOMA	X							X		X		X		X

	Year 1							Year 2						
Assessments	Screen	Basel.	Surg*	1st to 3rd PO *	W1*	W4	M3	M6	M9	M12	M15	M18	M21	M24
Meal tolerance test	X							X		X		X		X
Ionizable calcium	X							X		X		X		X
PTH	X							X		X		X		X
Vit D (25 OH)	X							X		X		X		X
Vit A	X							X		X		X		X
Folic acid	X							X		X		X		X
Vit B1	X							X		X		X		X
Vit B12	X							X		X		X		X
Anti-GAD	X													
Urine I	X									X				X
Urine culture (if necessary)														
PSA	X													
Anti-HIV	X													
Chest x-ray	X													
Abdominal ultrasound	X													
Electrocardiography	X													
Upper digestive endoscopy	X													
Echocardiogram	X													
Retinography	X							X		X		X		X
Funduscopy	X							X		X		X		X
Refraction test	X							X		X		X		X
Angiofluoresceinography (if necessary)	X							X		X		X		X
Vital signs	X		X	X	X	X	X	X	X	X	X	X	X	X
Weight	X				X	X	X	X	X	X	X	X	X	X
Height	X													
Drug dispensing	X		X	X	X	X	X	X	X	X	X	X	X	X
Gastric bypass			X											
Hospitalization			X											

* Applicable for patients randomized to the intervention group (surgical procedure).

7.6. ASSESSMENTS AND TESTS

Laboratory and imaging tests will be performed on the premises of Oswaldo Cruz Hospital and managed by Fleury Medicine and Health group, currently the largest center of diagnostic medicine in Brazil. The institution has the necessary national and international certifications, such as ISO 9001, ISO 14001, American College of Radiology, College of American Pathologists, Proficiency in Laboratory Tests of the Brazilian Society of Clinical Pathology/Laboratory Medicine, and the NGSP level I certificate of traceability to the DCCT reference method for assessment of metabolic parameters.

7.6.1. RENAL FUNCTION

It will be measured before and 6, 12, 18 and 24 months after inclusion in the trial. The glomerular filtration rate will be estimated using the Cockcroft-Gault equation and the Modification of Diet in Renal Disease (MDRD) equation.[34] Albuminuria will be measured and the albumin/creatinine ratio will be calculated in isolated urine samples collected in the morning. Patients will be instructed to avoid physical activity the day before the collection. Urine I will be collected at time points 0, 1 year and 2 years after inclusion in the trial, and if necessary (presence of leukocyturia), urine culture will be performed.

7.6.2. PERIPHERAL NERVOUS SYSTEM FUNCTION

The sensory and motor function of the peripheral nervous system will be assessed by nerve conduction studies (10-g monofilament and 128-Hz tuning fork)[33] before and 6, 12, 18 and 24 months after inclusion in the trial.

7.6.3. RETINAL ASSESSMENT

Retinopathy will be assessed and compared by retinography performed before and 6, 12, 18, and 24 months after inclusion in the trial. If indicated by the ophthalmologist, fluorescein angiography will be performed. This test can detect early changes in diabetic retinopathy.[35]

7.6.4. USE OF MEDICATIONS

All medications used will be clearly recorded, including trade and generic name, dose, and drug administration regimen. These data will be obtained by the researchers at each patient visit during follow-up. Treatment adherence will be determined based on the number of tablets and units of insulin given to the patient. At each visit, the patient will have to show to the researchers whether and how many tablets/units are left.

A scale of medication use will be calculated based on the number of medications and their dosage. For each oral medication, a numerical score will be calculated based on the ratio between daily and maximum recommended dose [UPTODATE, 2011]. In cases of insulin users, a numerical score will be calculated based on the ratio between daily insulin dose and the standard dose of 1U/kg/day. The final score will be obtained for each patient by the sum of the scores of each medication.

7.6.5. ASSESSMENT OF GLYCEMIC CONTROL

All patients will undergo measurement of fasting glycemia and HbA1c before and 3, 6, 9, 12, 15, 18, 21 and 24 months after inclusion in the trial. Fasting glycemia will also be measured one day, one week and one month after surgery. Patients will receive guidance regarding capillary glycemia performed 3 times a day for patients using insulin and twice a week for the other patients.

7.6.6. ASSESSMENT OF BLOOD PRESSURE

Blood pressure will be measured at each patient visit using a calibrated device, adjusted for arm circumference for each patient. The patient will be seated for at least 5 minutes before each measurement. An average of two measurements will be recorded.

7.6.7. ASSESSMENT OF LIPID PROFILE

Total cholesterol, LDL, HDL, and triglycerides will be measured in blood samples collected in the morning after a 10-hour overnight fast before and 6, 12, 18 and 24 months after inclusion in the trial.

7.6.8. ASSESSMENT OF QUALITY OF LIFE

Quality of life will be assessed by the SF-36 (Medical Outcomes Study 36 – Item short form health survey), translated and validated in Brazilian Portuguese [IQOLA SF-36, 1996], before and 12 and 24 months after inclusion in the trial.

7.6.9. ASSESSMENT OF TREATMENT COMPLICATIONS

All complications and adverse events will be clearly recorded and classified according to severity at each patient visit [CTCAE, 2009].

7.6.10. ESTIMATE OF MEDICATIONS AND CAPILLARY GLYCEMIA REAGENT STRIPS USED

Product	Surgical group (n=72)	Clinical group (n= 72)
Metformin 1g	4200 cp/month	4200 cp/month
Actos 30 mg	420 cp/month	630 cp/month
Linagliptin 5 mg	420 cp/month	630 cp/month
Liraglutide 6.0 mg/mL	28 pens/month	28 pens/month
Lantus	10500 IU/month	21000 IU/month
Apidra	1300 IU/month	5000 IU/month
Centrum	2100 cp/month	
Reagent strips	3100/month	3100/month

7.7. SAFETY ASSESSMENT

Safety will be assessed by clinical evaluations, laboratory tests, vital signs, and adverse events.

7.7.1. Adverse Events Assessment

Assessment of adverse events will begin upon signature of the informed consent form by the patient and will end 30 days after month-24 patient visit. If the patient withdraws from the trial before month-24 visit, adverse events will be collected for 30 days after the withdrawal date.

7.7.2. Definitions

7.7.2.1. Adverse Event

An adverse event is any unexpected medical occurrence in a research subject of a clinical trial. It does not necessarily imply any causal relationship with medications and materials used during the clinical trial or with the trial itself. An adverse event may be an unfavorable or unintended sign, symptom, syndrome or disease that develops or worsens during the clinical trial. Clinically relevant abnormal results of diagnostic procedures, including abnormal laboratory findings, are considered as adverse events.

Adverse events are categorized as "non-serious" and "serious" events.

7.7.2.2. Serious adverse event

A serious adverse event is an adverse event that results in any of the following outcomes:

- Death;
- Life-threatening situation;
- Hospitalization or prolongation of current hospital stay;
- Persistent or significant disability/deficiency;
- Congenital anomaly/birth defect;
- At the discretion of the Principal Researcher.

7.7.2.3. Recording and monitoring adverse events

Adverse events will be actively collected after the patient signs the informed consent form. Information collected should include diagnosis (based on signs and symptoms presented by the research subject), classification of event severity, start date, definition of the event causal relationship, medical practices, and event end date.

7.7.2.4. Definition of severity

All adverse events will be assessed (graded) regarding severity according to CTCAE version 4.0:

Grade 1 – mild adverse event
Grade 2 – moderate adverse event
Grade 3 – severe adverse event
Grade 4 – life-threatening or disabling adverse event
Grade 5 – death related to adverse event

7.7.2.5. Definition of drug-related adverse event

The relationship of adverse events with surgery, medications or procedures under study will be classified as follows:

- Unrelated;
- Improbable;
- Possibly related;
- Probably related;
- Definitely related.

7.7.3. Most Common Complications from Surgical Treatment

7.7.3.1. Early complications (within 30 days after surgery):

Intracavitary bleeding: originating in the surgical site or in the points of trocar insertion. Reintervention may be necessary for adequate hemostasis, with possible need for blood transfusion.

Gastrointestinal bleeding: resulting from the formation of acute ulcers of the digestive tract and/or from anastomosis sites. A new surgery may be necessary to control bleeding, with possible need for transfusion of blood components.

Anastomotic fistulas: duodeno-jejunal, entero-enteroanastomosis and gastroenteroanastomosis. The occurrence of fistulas may lead to the need for surgical treatment or even to the adoption of a conservative management. Occasionally, severe sepsis may occur, requiring prolonged hospitalizations. Surgical experience with similar procedures (bariatric surgery) indicates low rates of occurrence of this complication – 0.5/0.8% [BUCHWALD, 2004].

Gastrojejunal stenosis: occurring about 30 days after surgery. Its treatment often involves endoscopic dilatation.

Deep vein thrombosis: this event may occur in spite of the prophylaxis – use of low molecular weight heparin. The diagnosis will be made by Doppler imaging of the lower limbs. In the event of pulmonary embolism, the diagnosis will be established by CT angiography of the chest, and patients will be immediately admitted to the Intensive Care Unit.

Intestinal obstruction: this event is rarely observed in procedures performed by video laparoscopy. The initial treatment is clinical.

Other complications: Atelectasis; Pneumonia; Pancreatitis; Hematoma at the puncture site; Vomiting, nausea.

7.7.3.2. Late complications: Excessive weight loss and nutritional deficiencies.

Treatment of late complications after gastric bypass consists of protein and vitamin supplementation, by enteral or parenteral route in severe cases, such as Wernicke's encephalopathy, with good results, and no cases requiring surgical reintervention have been described.

7.7.4. Most Common Complications from Drug Treatment

7.7.4.1. Metformin

The most common adverse reactions with the use of metformin are gastrointestinal disturbances such as nausea, vomiting, diarrhea, and abdominal discomfort, as well as

malaise and hyperventilation. Lactic acidosis, sometimes fatal, was associated with treatment with metformin, however, almost all reported cases involved patients with contraindications to treatment or intake of excessively high doses. Occasionally, skin reactions and metallic taste may occur.

7.7.4.2. Pioglitazone

Although there are reports of heart failure and edema, pioglitazone appears to reduce triglyceride levels and to produce a significant increase in HDL cholesterol. There are differences in the modulation of the nuclear receptor expression of thiazolidinediones, which may explain a lower percentage of pioglitazone-related cardiovascular events compared to other drugs of the same pharmacological class.

Pioglitazone has been associated with bladder cancer and leiomyosarcoma. The PROspective pioglitAzone Clinical Trial In macroVascular Events (PROactive) recorded a significant marginal increase in the incidence of bladder cancer among those treated with pioglitazone (0.5%) compared to placebo (0.2%). The authors of the study related to the PROactive trial considered the events unrelated to the drug [CHARBONNEL, 2004].

Also, in the PROactive, an increase in the incidence of distal bone fractures was recorded in patients receiving pioglitazone (5.1%) compared to controls (2.5%) [CHARBONNEL, 2004].

Other drug-related reactions have been described, such as: sinusitis, headache, dental disorders, aggravated diabetes, and pharyngitis.

7.7.4.3. Linagliptin

Upper respiratory infections, cough, allergy, and (rarely) pancreatitis have been reported [SCHERNTHANER, 2012].

7.7.4.4. Liraglutide

Studies with rats have shown increased incidence of medullary thyroid carcinoma, but at doses much higher than those approved for use in human subjects. Nausea and vomiting are common, and rare cases of pancreatitis (7 cases among 4257 patients) have been described in phase 2 and 3 trials [PARKS, 2010].

7.7.4.5. Lantus

Hypoglycemia is the most common side effect, although it is less common in this type of slow-release insulin analog.

Allergic reactions and lipodystrophy may occur at the injection site.

7.7.4.6. Apidra

Hypoglycemia is the most common side effect. Allergic reactions and lipodystrophy may also occur at the injection site.

8. ETHICAL ASPECTS

8.1. ETHICAL AND REGULATORY CONSIDERATIONS

Patients will be included in the trial after formal authorization is provided in written by them by signing the informed consent form (APPENDIX 1), which will occur after adequate explanation of the nature of the project and after all questions of the patient have been answered, according to the Brazilian National Health Council Resolution No. 196, October 10, 1996. Upon agreement to participate in the trial, the patient will periodically receive reports on the disease progress, associated with full assistance from the research team, composed of: a general surgeon, an endocrinologist, and a nutritionist.

At the end of the study, the patients will be referred back to the medical service of origin for monitoring of their disease.

8.1.1. REGULATIONS

This trial will be conducted in accordance with the research protocol, Good Clinical Practices, ethical principles of the Declaration of Helsinki, and the Brazilian legislation that regulates clinical research (Resolution 196/96 and complementary provisions).

The Principal Researcher will submit to the IRB written reports on the status of the clinical trial every six months. A final trial report will also be sent to the IRB after the completion of the trial or in the case of early termination of the trial, in accordance with the applicable rules. Copies of all contact with the IRB and the National Ethics Committee will be filed together with all documents related to the clinical trial.

8.1.2. ETHICAL APPROVAL

Before the start of the trial, the protocol will be submitted to the IRB. The trial will only start after approval of the IRB, and if applicable, of the National Ethics Committee. Any amendments to the protocol or to the informed consent form should be previously approved by the IRB prior to their implementation. The only exception is when the procedures/changes pose risks to the safety of research subjects if not performed; in this situation, the Principal Researcher will implement the changes and immediately notify the IRB.

8.1.3. REIMBURSEMENT OF RESEARCH SUBJECTS

Research subjects will be reimbursed for expenses related to the trial. Trial-related expenses are defined as expenses that involve transportation and meals. Research subjects will not receive any payment for participation in the clinical trial, in accordance with Brazilian law.

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