Journal of Clinical and Molecular Medicine

Research Article ISSN: 2516-5593

Evaluation of type 2 diabetes mellitus remission and oxidative stress profile after Laparoscopic Sleeve Gastrectomy (LSG) in Romanian obese male patients

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Abstract

Background: Geography is one of the key drivers of the significant variation in the etiopathogenic profile and prevalence of diabetes and obesity, highlighting the need for local studies to fundament the most appropriate interventions. Presently, the criteria for choosing the candidates with T2DM and obesity who will benefit most from laparoscopic sleeve gastrectomy (LSG) have not reached a worldwide consensus, supporting the current need for sharing experts' guidance in the preoperative evaluation, choice of interventional procedure, perioperative management and patients' long-term care. The aim of the current study was to evaluate the impact of LSG on T2DM remission in Romanian obese male patients, based on a multiparametric, prospective investigation.

Methods: We have conducted a randomized controlled study on 41 obese male participants (body mass index, BMI ≥ 30 kg/m2), with type 2 diabetes mellitus (T2DM), aged 30 - 65 years, which were randomly divided in 2 study groups: one receiving conventional treatment and the second undergoing LSG. The clinical and anthropometrical parameters, resting metabolic rate, general biochemical status, adipocyte profile, gastrointestinal hormones levels, pro-inflammatory, oxidant and antioxidant profiles were determined at three time points: V1 (baseline), V2 (after 6 months) and V3 (after 12 months).

Results: The LSG impacted more significantly than the conventional treatment the following parameters: glycated haemoglobin (HbA1c) (89% versus 14%), blood glucose levels, BMI, weight, visceral fat level, HDL-cholesterol, incretin hormones, pro-inflammatory and the oxidative stress status.

Conclusions: This is the first study reporting on the evaluation of metabolic surgery impact on Romanian obese male patients. Our results confirm that LSG could increase the chance for T2DM remission as compared with standard medical therapy, in patients with diabesity. The duration of T2DM seems to be a more critical factor than the patient's obesity itself, in selecting patients with T2DM for surgery. In addition, age, BMI and C Peptide parameters, already included in the prediction algorithm, the proinsulin levels, proinsulin / insulin ratio and the visceral fat percentage proved also to be valuable markers for monitoring the disease.

Introduction

Presently, we are facing a worldwide epidemic of obesity and T2DM mellitus (T2DM), which are often linked together, as suggested by the currently used term of "diabesity" [1]. The most recent global predictions of the International Diabetes Federation (IDF) suggest that there are 285 million people with diabetes currently worldwide. This is set to escalate to 438 million by 2030 [2], with a further half billion at high risk, diabetes being therefore one of the greatest public health threats of the 21st century. Premature mortality and morbidity in diabetes mainly result from microvascular and macrovascular complications, but also from other dysfunctions involving lipid metabolism, oxidative stress and inflammation. On the other hand, studies have shown that untreated obesity can shortly lead to diabetes. The excessive adipose tissue is associated with a chronic pro-inflammatory condition, contributing to the occurrence of insulin resistance, a fundamental pathogenic mechanism involved in the development of T2DM.

Current data highlight the fact that, In Romania, due to medical, social, dietary, emotional or hereditary factors, the number of "diabesity" cases is alarmingly increasing, nearly 2 out of 10 Romanian youngsters aged between 15 and 24 being overweight [3,4] and 20% of Romanian people with class 1 and 2 obesity or with morbid obesity

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Key words: *T2DM, laparoscopic sleeve gastrectomy, diet, conventional antidiabetic oral therapy, obesity, oxidative stress, incretin hormones, pro-inflammatory state*

Received: January 18, 2020; **Accepted:** April 25, 2020; **Published:** April 29, 2020

(class 3) develop T2DM. According to the PREDATORR (Prevalence of diabetes mellitus and prediabetes in the adult Romanian population) study conducted in 2016, most cases of T2DM that are encountered among Romanian people are associated with morbid obesity, the percentage being about 48% [4].

The term 'bariatric' surgery, derived from the Greek word "baros"(for weight), subsequently changed to bariatric–metabolic surgery defines the surgical procedures designed to produce substantial weight loss, associated with other significant health benefits, including improvement or normalization of hyperglycaemia, hyperlipidaemia, blood pressure, obstructive sleep apnea and improved quality of life [5,6], being presently considered an efficient tool for both T2DM control and remission [7]. There are many surgical interventions that can be grouped in three main categories, i.e. blocking, restricting, and mixed procedures [8]. Laparoscopic sleeve gastrectomy (LSG) is an emerging bariatric surgery procedure with increasing popularity, consisting in the resection of the main part (about 75%) of the fundus and corpus of the stomach, starting 2-8 cm proximally to the pylorus and leaving a narrow gastric tube or "sleeve", without bypassing or removing intestines [9,10]. The option of bariatric intervention needs to be carefully considered in appropriately selected individuals.

Despite a number of evidence-based reviews and consensus statements having been published regarding the use of bariatric surgery in patients with obesity and diabetes [11], there is still need for studies reporting on the worldwide expert guidance in the preoperative evaluation, choice of interventional procedure, perioperative management and long-term care of patients seeking surgery to improve diabetes control. The IDF Taskforce on Epidemiology and Prevention convened a consensus working group of diabetologists, endocrinologists, surgeons and public health experts in December 2010, to discuss the appropriate role of bariatric surgery and of other gastrointestinal interventions in the treatment and prevention of obesity and T2DM.

The aim of the current study was to evaluate the impact of LSG on male patients with T2M and obesity, by performing a multiparametric, prospective investigation of clinical and anthropometrical parameters, resting metabolic rate, general biochemical status, adipocyte profile, gastrointestinal hormones levels, pro-inflammatory, oxidant and antioxidant profile, including respiratory burst determination in peripheral blood mononuclear cells, all determined before, as well as 6 months and 12 months after the LSG intervention.

Experimental Section

Materials and Methods

We conducted a randomized controlled study on 41 T2DM obese male participants (body mass index, BMI ≥ 30 kg/m^2), which were randomly selected from a pool of 144 Caucasian patients from all over the country, aged 30 - 65 years. The study was approved by the local Ethics Committee from "Ponderas" Hospital and "Prof. N. C. Paulescu" National Institute of Diabetes, Nutrition and Metabolic Disease, Bucharest. All patients participating in the study signed an informed consent in accordance with the Helsinki Declaration (1975, revised in 2008), and procedures for working with human subjects and biological samples from them received the favourable opinion of the Ethics Committees of each partner institution. Due to the high cost of LSG surgery in Romania, only a limited number of subjects could be included in our study. Taking this into account, we decided to analyse only male subjects with the same type of obesity (predominantly abdominal obesity), in order to achieve a homogeneous study group. All 41 patients were randomly divided into two groups: (1) patients receiving conventional (non-surgical, antidiabetic) treatment of T2DM and (2) patients undergoing LSG. The randomization of patients included in the study is based on a pseudo-generator created on the Microsoft Visual Studio 2013 Community Edition platform, based on the algorithm described by Donald E. Knuth [12].

The following inclusion criteria were used: sex- male, age 30 - 65 years, T2DM 1-15 years duration, body mass index (BMI) between 30-50 kg/m^2 (all subjects had abdominal obesity), possibility of covering the cost of post-operative medication after LSG.

The exclusion criteria were: type 1 diabetes, C-peptide < 0.81 ng/mL, HbA1c <6.5%, anemia (Hb < 10 g/dL), the presence of active liver disease or hepatic dysfunction (hepatitis B or C, cirrhosis), renal disease (serum creatinine >1.2 mg/dL or glomerular filtration rate GFR < 60 mL/min/1.73 m^2), malignancies, coronary artery disease with myocardial infarction or stroke in the last 12 months, thyroid and psychiatric pathology, chronic pathology of the digestive tract or the adjacent glands and/or major surgical interventions in the digestive system (gastric / intestinal resections / acute pancreatitis), smoking, alcoholism, drug dependence. No participant had any diagnosed systemic immune disorder and was not known to be taking any form of vitamin supplementation neither at the time of recruitment nor after inclusion in this study, or any other treatment with immunosuppressant, corticosteroids and anticoagulant therapy.

Starting from the measured value for resting metabolic rate (RMR) and taking into account the "nutritional pattern" or identified dietary habits, a personalized diet was established for each patient from the conservative treatment group [13]. Specifically, the daily caloric requirement was calculated based on the formula [RMR*1.3 (sedentary lifestyle) + (10% * RMR) (for the thermal effect of food) – 500], thus inducing a 500-kcal daily restriction. In addition, all subjects received lifestyle counselling regarding the increase of physical activity (≥30 minutes of brisk walking every day, or moderate exercise at least 30 min, 3 to 5 times per week), limiting alcohol consumption and cessation of smoking, maintaining and monitoring the prescribed oral therapy for T2DM with metformin (1-3 g/daily), hypertension and dyslipidaemia according to current guidelines. Unfortunately, with extremely rare exceptions, clinically significant weight loss is generally very modest and transient, particularly in patients with severe obesity [14,15]. The failure rate for these programs was around 95% after 1 year.

Patients from the surgical group underwent an LSG procedure. Subsequently, they received specific dietary advice (vitamins and minerals supplementation) and down-titration of diabetes medication according to their blood glucose profiles and even removal of antidiabetic oral medication. Patients in the two groups were followed for over one year to observe the impact of LSG in comparison with the group receiving conventional treatment on decreasing body fat mass and main parameters of glucose, lipid and protein metabolism. Based on this multiparametric investigation we were able to particularize the selection algorithm of patients with T2DM and obesity having the highest chances of diabetes remission after correcting obesity taking into account the gropgraphical variation.

Before LSG, at visit V1 (considered the starting point of the study) and then on the occasion of the follow up visits, i.e. V2 (after 6 months) and V3 (after 12 months), the below investigations were carried out for all 41 patients.

A complete physical examination was performed and information was gathered about: age, height, weight, abdominal circumference, heart rate, blood pressure, clinical examination of the respiratory, digestive system, clinical information about treatment, duration of T2DM, eating habits and life style habits.

For all patients, the body composition was determined by the bioimpedance method (using the Body Composition Analyzer Tanita BC-418 MA), thus determining the following parameters: weight, body mass index (kg/m^2), body fat (%), fat mass (kg), free fat mass (kg), total body water (kg), visceral-fat rating (%).

The resting metabolic rate (RMR) was determined by indirect calorimetry with the COSMED QUARK CPET analyzer (Cardio Pulmonary Exercise Testing). In diabetes with obesity, RMR is higher than in obesity alone, due to increased glucose oxidation, decreased glucose storage and increased sympathetic nervous system activity [16].

Blood samples were collected for the following laboratory investigations: baseline biochemical measurements, HbA1c (by high performance liquid chromatography - HPLC method on D10-BioRad Analyser), blood count (by automatic photometric method on Cell-Dyn 3700 BioRad Analyser), rapid serological tests for HIV, hepatitis B and C. glycaemia, a lipidic (total serum cholesterol, high density lipoprotein HDL-cholesterol, low density lipoprotein LDL-cholesterol, serum triglycerides), and also a hepatic and renal profile (creatinine, urea, uric acid, albumin, total protein, hepatic transaminases and gamma glutamyl transferase GGT) was performed on the EOS BRAVO FORTE HOSPITEX DIAGNOSTICS biochemistry analyzer using specific reagents according to the manufacturer's technical datasheets.

Serum insulin, proinsulin, C peptide were determined by Enzyme-Linked Immunosorbent Assay (ELISA) using commercially available kits (EIA-2935, EIA-1560, EIA-1293, DRG Instruments, Germany). Absorbance reading (at 450 nm) was performed on ELISA plate reader: MULTISKAN Ex-Thermo Electro Corporation (CV = 2.6%).

Based on blood glucose and insulinemia values, beta cell function was estimated (HOMA% B - Homeostasis Model Assay for β cell function) according to the formula (20 x fasting insulin (μU/mL)/ (fasting blood glucose (mmol/L)-3.5%. Also, insulin resistance was assessed by calculating HOMA-IR (Homeostasis Model Assay for Insulin Resistance) by the formula: [(fasting blood glucose (mmol/L) x insulinemia (μU/mL)]: 22.5.

Leptin and adiponectin hormones (used to evaluate adipocyte cell function) have also been determined by the Enzyme-Linked Immunosorbent Assay (ELISA), using the commercially available kits EIA-2395 and EIA-4177, and the DRG Instruments (Germany), according to the manufacturer's recommendations. Proinsulin / Adiponectin (P/A) and Proinsulin / Insulin (P/I) ratio was determined by mathematical formula.

Concentrations of hormones involved in the regulation of food intake, including Glucagon Like Peptide 1 (GLP-1) with anorexic role and ghrelin with orexigenic role, have also been determined by using commercially available ELISA kits (MBS760336 MyBioSource, INC., Biozyme for GLP-1 kits and EIA-4710 kit for the active (acylated) form, Human Ghrelin (active) (DRG Instruments, Germany).

Tumor necrosis factor alpha (TNF-α), interleukin 6 (IL-6), high sensitivity C reactive protein (hsCRP) and homocysteine circulating pro-inflammatory markers were determined by commercially available ELISA kits EIA-4641, EIA-4640, EIA-3954 (DRG Instruments, Germany) and MBS260128 MyBioSource INC (Biozyme).

For all ELISA tests, absorbance reading (at 450 nm) was performed on the automated ELISA plate reader: MULTISKAN Ex-Thermo Electro Corporation (CV = 2.6%).

The oxidative stress profile consisting in the evaluation of "Respiratory Burst" and the antioxidants enzymes: paraoxonase1 (PON1), superoxide dismutase (SOD) , glutathione peroxidase (GPx) ,catalase (CAT) and also 8-OH-2-deoxiguanosine – marker for the oxidative modification of DNA was investigated at the V1 and V2.

Evaluation of oxidant / antioxidant status in the peripheral circulation of patients with obesity and T2DM involved isolation of peripheral blood mononuclear cells (PBMC) and respiratory burst (RB) determination in all patients.

The ability of PBMCs to produce free radicals was determined by measuring the activity of NADPH oxidase Nox2. Mononuclear cells in the peripheral blood were isolated by density gradient centrifugation with Ficoll-PaqueTM Plus (1.0077g/ml). After centrifugation at 630g for 30 minutes the PBMCs were harvested, washed twice, and resuspended in 1 ml phosphate buffer saline (PBS). Cell viability was checked with Trypan Blue and was always ≥ 90%. The RB production was monitored by assessing the lucigenin (LG) / luminol (LM) chemiluminescence on PMA stimulation (phorbol-12 myristate-13 acetate) / opsonized zymozan (OZ). Over the isolated PBMCs (0.3x106 cells) resuspended in PBS, lucigenin and luminol were added. Spontaneous chemiluminescence monitoring was performed for 15 minutes, after which RB was initiated by the addition of PMA / OZ, and the chemiluminescence peak was recorded with the LUMINOSKAN ASCENT THERMO SCIENTIFIC luminometer. The results were expressed as Relative Chemiluminescence Units (RLU).

Determination of erythrocyte glutathione was performed on total blood samples (200 μl) after precipitation with metaphosphoric acid solution, disodium salt dihydrate (Na2EDTA) and sodium chloride (NaCl). After centrifugation at 4000 rpm, over 250 μl of the supernatant was added 1 ml of 0.3 M phosphate buffer and 125 μl DTNB (Ellman reagent: 5,5'-dithiobis-2-nitrobenzoic acid) over 15 minutes at 4°C. Absorbance was read at 405 nm, and the results were expressed as μg/g Hb.

Antioxidant enzymes PON1, arylesterase (PON1phe) and lactonase 1 (PON1dhe) activities were measured toward 1 mM phenylacetate in 20 mM Tris/HCl pH 8.0 or 1 mmol/L DHC respectively. The reaction was started by the addition of the serum and the increase in absorbance was read at 270 nm using a UV–VIS spectrophotometer. Blanks were included to correct the spontaneous hydrolysis of substrate. One unit (U) of arylesterase (PON1phe) is defined as 1 pmol of p-nitrophenol hydrolysed per minute using the extinction coefficient of 1310 M-1 cm-1 while one unit of lactonase activity (PON1dhe) is equal to 1μmol of DHC hydrolysed/mL/min using the extinction coefficient of 1295 $M^{-1}cm^{-1}$. The intra- and inter-assay coefficients of variation were < 5% in all tests.

Superoxide Dismutase (SOD) and Glutation Peroxidase (GPx) were measured by using kit no. K9120 for SOD and kit no. 30-7031 for GPx, respectively (Sigma-Aldrich Co. LLC., St Louis, USA), according to the producer recommendations. Results are expressed as U/g Hb. The method of determining the activity of erythrocyte catalase (CAT) is based on the decrease in the absorbance of H_2O_2 at 240 nm with decreasing concentration. The initial haemolysate obtained was diluted with 50 mM phosphate buffer (pH 7.0), and the reaction was initiated with H_2O_2. The results were expressed in k/g Hb.

Determination of the serum 8-OH-2- deoxiguanosine (8-OH-2dG) concentrations was performed with a competitive ELISA kit (Abcam) using a monoclonal antibody specific for 8-OH-2dG and an acetylcholin-esterase (AChE) conjugated 8-OH-2-dG tracer according to the manufacturer instructions; the substrate for AChE is contained in the developing reagent, and the yellow enzymatic reaction product was determined spectrophotometrically at 412 nm.

To avoid possible postoperative complications, in addition to the conventional treatment group, patients randomized to the surgical treatment group have undergone preoperative investigations: barium transit, upper digestive endoscopy under intravenous anesthesia, cardiac evaluation, spirometry, abdominal ultrasound scan, chest X-ray.

Out of the 21 patients in the conventional group and 20 patients in the surgical group initially included in the study, 19/17 reached V2 and 19/15 patients were present at the last visit (therefore five patients decided to withdraw from study).

Statistical analysis

All statistical analyses were conducted using the SPSS version 20.0 software. We used mean ± standard deviation (SD) / standard error of mean (SEM) to describe continuous variables with a normal distribution and median with interquartile range (in brackets) for variables with skewed distribution. Some variables were converted logarithmically to normalize their distribution before analysis. Results for variables that did not follow a normal distribution were presented as median and interquartile range (IQR) (25-75). Paired Student's t-tests and Wilcoxon signed rank test were used to compare data from the two groups. A value of $p < 0.05$ and $p < 0.001$ was considered statistically significant.

Results

This is the first prospective randomized study of the remission potential of T2DM after the bariatric-metabolic surgery in comparison with conventional treatment performed on Romanian patients.

There were no statistically significant differences between the parameters determined at baseline for the two studied groups, which demonstrates the validity of the randomization algorithm used in our study. Overall patients were relatively young, with a mean age for conventional treatment group of 48.7 ± 6.8 years and of 46 ± 5.9 for the LSG group, with morbid obesity and poor metabolic control for both groups, despite the relatively short duration of T2DM, i.e. 6.3 ± 4.5 years for the conventional group and 5.4 ± 2.9 for the LSG one. Table 1 summarizes the results of the clinical, anthropometrical, nutritional and metabolic status evaluation of the patients from the two study groups at the beginning of the study (V1).

The results of the multiparametric investigation for both studied groups obtained at V1 were used as reference and compared to those obtained at V2 (Table 2) and V3 respectively (Table 3).

Patients from the conventional group showed a statistically significant (but with minor clinically significant impact) decrease for BMI and waist circumference, as well as a significant improvement in systolic blood pressure values following a change in the dietary

Table 1. Results of Clinical, Biochemical, Anthropometrical, Nutritional and Metabolic Status Parameters Determination in the Two Studied Groups at Baseline (V1)

Clinical and Biochemical Parameters	Conventional Treatment Group (CTG)	LSG Group	P value
	n = 21	n = 20	
Waist Circumference (WC) (cm)	135.9 ± 10.72	139.85 ± 16.69	0.37
BMI (kg/m^2)	41.51 ± 5.56	41.2 ± 4.8	0.85
Systolic Blood Pressure (SBP) (mmHg)	135.29 ± 13.59	131.58 ± 16.33	0.43
Diastolic Blood Pressure (DBP) (mmHg)	83.8 ± 8.08	76.05 ± 11.37	0.17
HbA1c (%)	8.35 ± 1.49	8.82 ± 1.56	0.33
Glycaemia (mg/dl)	213.19 ± 84.17	220.9 ± 90.33	0.77
Insulin (μUI/mL)	28.41 ± 3.76	23.71 ± 3.74	0.38
HOMA-IR (%)	14.48 ± 5.47	14.88 ± 5.88	0.82
HOMA-β (%)	129.77 ± 63.07	132.79 ± 85.76	0.89
Proinsulin (pmol/L)	11.72 ± 2.25	7.67 ± 1.86	0.21
Proinsulin/Insulin	0.46 ± 0.09	0.37 ± 0.06	0.46
C Peptide (ng/ml)	9.40 ± 0.47	9.36 ± 0.46	0.95
Total cholesterol (mg/dl)	203.2 ± 33.41	182.49 ± 36.40	0.06
HDL-cholesterol (mg/dl)	37.19 ± 6.36	34.62 ± 13.67	0.44
Triglycerides (mg/dl)	211.10 ± 92.51	218.94 ± 117.77	0.81
LDL-cholesterol (mg/dl)	123.79 ± 33.8	104.08 ± 39.70	0.09
Uric Acid (mg/dl)	5.65 ± 2.05	5.99 ± 1.84	0.58
Creatinine (mg/dl)	1.10 ± 0.24	1.13 ± 0.20	0.77
Urea (mg/dl)	39.74 ± 20.82	40.55 ± 7.58	0.87
AST	36.02 ± 4.45	35.89 ± 4.82	0.98
ALT	65.03 ± 15.24	47.43 ± 6.19	0.3
GGT	106.42 ± 38.16	59.52 ± 8.15	0.24
Albumin (g/dl)	4.55 ± 0.39	4.44 ± 0.32	0.35
Total Protein (g/dl)	7.22 ± 0.54	7.1 ± 0.59	0.49
RMR measured (kcal/day)	2381.2 ± 462.15	2512.7 ± 440.25	0.36
RMR predicted (kcal/day)	2438.5 ± 285.7	2413.05 ± 254.98	0.76
VO2 in rest state (ml/min)	347.6 ± 68.19	364.4 ± 62.82	0.42
VCO2 in rest state (ml/min)	272.85 ± 52.64	295.35 ± 57.19	0.2
Fat Mass (%)	35 ± 5.32	31.12 ± 8.74	0.09
Free Fat Mass (kg)	81.9 ± 13.85	87.92 ± 14.39	0.18

Table 2. Comparison of clinical and metabolic characteristics determined at V1 and V2 for the two study groups

Clinical and Biochemical Parameters	CTG		P	LSG		P
	V1	V2		V1	V2	
	n=21	n=17		n=20	n=19	
Waist Circumference (cm)*	139.0 (14.0)	133.00 (10.00)	0.001302[1]	134.0 (16.0)	105.0 (10.5)	0.0001406[1]
BMI (kg/m²)*	40.50 (8.60)	39.10 (8.30)	0.0004803[1]	39.60 (4.90)	29.30 (2.25)	0.0001428[1]
SBP (mmHg)*	135.0 (12.0)	120.00 (10.00)	0.04345[1]	130.00(13.75)	125.0 (30.50)	0.3256[1]
DBP (mmHg)*	90.00 (10.00)	80.00 (20.00)	0.262[1]	80.00 (10.00)	82.00 (13.00)	0.03823[1]
HbA1c (%)*	7.56 (1.87)	7.91 (2.62)	0.5791[1]	8.40 (1.45)	6.60 (0.75)	< 0.000001[1]
Glycaemia (mg/dl)*	184.5 (99.3)	145.17 (103.63)	0.2842[1]	197.7 (107.4)	99.56 (27.48)	< 0.000001[1]
Total cholesterol (mg/dl)#	194.14 ± 26.75	191.16 ± 55.01	0.08558[2]	184.65 ± 36.04	184.55 ± 32.05	0.99[2]
HDL-cholesterol (mg/dl)*	36.10 (8.20)	35.30 (9.50)	0.2446[1]	34.20 (8.15)	39.20 (9.90)	0.01236[1]
Triglycerides (mg/dl)*	187.4 (123.26)	167.35 (100.68)	0.579[1]	178.23 (137.50)	89.43 (48.30)	<0.000001[1]
LDL-cholesterol (mg/dl)*	121.15 (31.6)	119.86 (42.86)	0.2247[1]	111.15 (34.7)	126.54 (17.5)	0.06629[1]
Uric Acid (mg/dl)*	5.99 (1.45)	6.38 (1.54)	0.1594[1]	5.89 (2.34)	6.51 (2.19)	0.2753[1]
Creatinine (mg/dl)*	1.02 (0.10)	1.10 (0.22)	0.9058[1]	1.09 (0.19)	1.00 (0.15)	0.02166[1]
Urea (mg/dl)*	35.25 (7.12)	33.92 (8.19)	0.7119[1]	40.18 (9.31)	36.93 (11.07)	0.8596[1]
AST (IU/L)*	24.34 (12.45)	20.51 (14.75)	0.07968[1]	29.60 (23.58)	16.74 (3.68)	< 0.000001[1]
ALT (IU/L)*	36.18 (17.38)	33.06 (24.66)	0.1743[1]	41.38 (46.41)	15.36 (6.16)	< 0.000001[1]
GGT (IU/L)*	45.34 (24.00)	33.46 (13.15)	0.07968[1]	53.91 (40.11)	28.57 (22.34)	0.002838[1]
Albumin (g/dl)*	4.59 (0.43)	4.55 (0.28)	0.3087[1]	4.48 (0.32)	4.58 (0.23)	0.4444[1]
Total Protein (g/dl)*	7.28 (0.74)	7.33 (0.45)	0.246[1]	7.27 (0.82)	7.08 (0.65)	0.7475[1]

* - Median and IQR range; # - mean ± SD; [1] - Wilcoxon signed rank test; [2] - Paired T Test

Table 3. Comparison of clinical and metabolic characteristics determined at V1 and V3 for the two study groups

Clinical and Biochemical Parameters	CTG		P value V1 vs. V3	LSG		P value V1 vs. V3
	V1	V3		V1	V3	
	n=21	n=15		n=20	n=19	
Waist Circumference (cm)*	139.0 (14.0)	128.00 (13.00)	0.000714[1]	134.0 (16.0)	102.0 (10.25)	0.0001421[1]
BMI (kg/m²)*	40.50 (8.60)	40.30 (7.50)	0.002851[1]	39.60 (4.90)	28.70 (1.50)	< 0.000001[1]
SBP (mmHg)*	135.0 (12.0)	130.00 (19.00)	0.8504[1]	130.00(13.75)	128.0 (31.50)	1.000[1]
DBP (mmHg)*	90.00 (10.00)	92.00 (11.00)	0.1236[1]	80.00 (10.00)	86.00 (22.00)	0.02929[1]
HbA1c (%)*	7.56 (1.87)	8.74 (2.69)	0.804[1]	8.40 (1.45)	5.90 (0.69)	0.0001426[1]
Glycaemia (mg/dl)*	190.1 (168.0)	163.80 (119.97)	0.1354[1]	197.7 (107.4)	96.33 (29.98)	< 0.000001[1]
Total cholesterol (mg/dl)#	208.91 ± 37.66	196.06 (± 44.23)	0.03876[2]	184.65 ± 36.04	188.75 ± 41.84	0.7105[2]
HDL-cholesterol (mg/dl)*	35.70 (9.60)	32.40 (15.45)	0.9547[1]	34.20 (8.15)	50.70 (15.65)	< 0.000001[1]
Triglycerides (mg/dl)*	201.10 (116.85)	147.62 (111.41)	0.05536[1]	178.23 (137.50)	86.26 (64.14)	< 0.000001[1]
LDL-cholesterol (mg/dl)*	121.15 (31.6)	130.43 (50.94)	0.804[1]	111.15 (34.7)	111.4 (61.53)	0.2935[1]
Uric Acid (mg/dl)*	5.99 (1.45)	5.96 (1.92)	0.7197[1]	5.89 (2.34)	5.59 (1.34)	0.9217[1]
Creatinine (mg/dl)*	1.02 (0.10)	1.04 (0.22)	0.164[1]	1.09 (0.19)	1.02 (0.12)	0.06409[1]
Urea (mg/dl)*	35.25 (7.12)	37.84 (11.62)	0.5995[1]	40.18 (9.31)	49.88 (14.72)	<0.000001[1]
AST (IU/L)*	24.34 (12.45)	22.10 (9.53)	0.2293[1]	29.60 (23.58)	17.38 (5.32)	<0.000001[1]
ALT (IU/L)*	36.18 (17.38)	30.52 (12.71)	0.2293[1]	41.38 (46.41)	22.80 (9.63)	0.003918[1]
GGT (IU/L)*	45.34 (24.00)	33.93 (8.83)	0.1876[1]	53.91 (40.11)	30.95 (20.36)	0.002022[1]
Albumin (g/dl)*	4.59 (0.43)	4.62 (0.31)	0.3626[1]	4.48 (0.32)	4.54 (0.30)	0.7022[1]
Total Protein (g/dl)*	7.28 (0.74)	7.29 (0.55)	0.4212[1]	7.27 (0.82)	7.11 (0.74)	0.5066[1]
RMR measured (kcal/day)*	2218 (557.50)	2073 (274.00)	0.8926[1]	2489 (471)	1732 (276)	<0.000001[1]
RMR predicted (kcal/day)*	2413.43 ±276.73	2372.84 (± 286.59)	0.003198[2]	2392.31 ± 244.03	1846.31 ± 175.58	<0.000001[2]
RMRm - RMRp	-118.68 ±412.26	-150.38 ± 356.41	0.6981 [2]	119.89 ± 396.91	-125.00 ± 170.52	0.009787 [2]
Fat Mass (%)*	36.50 (7.80)	34.80 (7.65)	0.03087 [1]	32.70 (9.85)	20.30 (7.60)	<0.000001[1]
Free Fat Mass (kg)*	77.40 (10.50)	81.10 (10.25)	0.01703 [1]	86.96 (20.50)	71.50 (9.60)	<0.000001[1]
Visceral Fat Level (%)#	20.86 ± 5	19.2 ± 4.92	0.331	21.65 ± 5.28	10 ± 4.07	<0.001

* - Median and IQR range; # - mean ± SD; [1] - Wilcoxon signed rank test; [2] - Paired T Test

habits. In contrast, patients from the surgical group showed statistically significant and clinically relevant improvements for most clinical and biological parameters, confirming the therapeutic efficacy of this surgical procedure.

Analysis of the results recorded with the occasion of the last visit (V3) indicated that patients from the surgical group continued to experience significant improvements in BMI, waist circumference, metabolic control, and lipid parameters.

As shown in Table 3, patients in the conventional group harbored a statistically significant decrease only for baseline metabolism (RMR) predicted on the basis of calculation equations (and explained by the concurrent modification of BMI), while patients in the surgical group

presented significant changes for both predicted RMR and the one actually measured by indirect calorimetry.

Table 3 also reveals a decrease in fat mass percentage for patients in both groups, more significant in the surgical group, as expected. In the surgical group, a decrease in weight, along with a decrease in BMI was recorded, while patients from the conventional treatment group showed a paradoxical weight gain, probably due to the loss of motivation to respect treatment, diet and physical exercise recommendations on long-term.

Concerning the hormonal levels (Tables 4 and 5), patients in both groups showed significant decreases of leptin level, in parallel with the decrease in BMI. Patients in the surgical group showed a significant decrease in circulating levels of ghrelin, the orexigenic hormone, probably due to the weight loss, while patients in the conventional group experienced a significant increase in this hormone. In addition, the patients in the surgical group experienced a significant increase in adiponectin (probably contributing to the improvement of insulin resistance) and a decrease in GLP-1.

Patients in the surgical group exhibited a statistically significant decrease in HOMA-IR, C peptide levels and proinsulin / insulin ratio - an indirect indicator of the degree of beta-cell dysfunction, pointing an improvement in glycemic control in these patients (Table 6). In addition, the significant decrease in circulating levels of leptin and ghrelin 6 and 12 months after surgery was correlated with a substantial decrease in BMI in these patients. For conventional treatment patients, these parameters did not show statistically significant differences between V1 and V3, except for the lower levels of proinsulin recorded at V3, as compared to V1 and V2.

Concerning the pro-inflammatory status, in the surgical group, both IL-6 and TNFα exhibited post-pacing increased values at 6 months (V2) with a stabilization / slight decrease at 12 months (V3). For the conventional group, the evolution was similar, with an increase noticed at V2 and stabilization at V3.

Gastric sleeve intervention resulted in a significant decrease in subclinical inflammation in parallel with weight loss, as indicated by hsCRP values. The decrease was significant after 6 months and continued 12 months post- surgery. For the conventional group

Table 4. Comparison of the hormonal levels determined at V1, V2 and V3 for the conventional treatment group (CTG)

Hormones	CTG				
	V1	V2	P value V2 vs. V1	V3	P value V3 vs. V1
	n=21	n=17		n=15	
Insulin(μUI/mL)*	22.12 (23.41)	17.43 (12.71)	0.07141[1]	14.13 (31.83)	0.2078[1]
HOMA-IR*	8.12 (14.02)	7.35 (5.70)	0.06383[1]	6.32 (11.93)	0.1688[1]
HOMA-β*	90.68 (52.24)	86.76 (131.14)	0.3529[1]	67.54 (146.79)	0.7615[1]
Proinsulin (pmol/L)*	6.42 (19.76)	4.84 (6.60)	0.2435[1]	3.06 (5.31)	0.00116[1]
Proinsulin/Insulin*	0.44 (0.59)	0.21 (0.73)	0.1202[1]	0.19 (0.15)	0.0946[1]
C Peptide (ng/ml)*	9.82 (2.53)	7.66 (2.10)	9.82 (2.53)	14.13 (31.83)	0.2078[1]
Leptin (ng/ml)*	15.50 (7.66)	10.16 (12.78)	0.005569[1]	8.62 (7.45)	0.00262[1]
Adiponectin (μg/ml)*	4.82 (9.28)	3.98 (6.50)	0.06641[1]	3.52 (2.61)	0.0832[1]
GLP-1 (ng/ml)*	47.13 (4.85)	50.54 (9.11)	0.5171[1]	-	-
Ghrelin (pg/ml)*	100.47 (47.2)	136.75 (62.89)	0.02016[1]	140.48 (115.6)	0.0946[1]

*median and IQR (in brackets); [1]Wilcoxon signed rank test; [2]paired T test

Table 5. Comparison of the hormonal levels determined at V1, V2 and V3 for LSG group

Hormones	LSG				
	V1	V2	p V2 vs. V1	V3	p V3 vs. V1
	n=20	n=19		n=19	
Insulin(μUI/mL)*	18.92 (24.35)	6.67 (5.68)	< 0.000001[1]	5.08 (4.17)	< 0.000001[1]
HOMA-IR*	8.44 (10.56)	1.68 (1.70)	< 0.000001[1]	1.19 (0.80)	< 0.000001[1]
HOMA-β*	60.16 (84.72)	72.03 (97.63)	0.04937[1]	61.25 (49.27)	0.5412[1]
Proinsulin (pmol/L)*	5.86 (6.59)	0.87 (0.71)	< 0.000001[1]	0.70 (0.87)	< 0.000001[1]
Proinsulin/Insulin*	0.32 (0.29)	0.12 (0.12)	< 0.000001[1]	0.12 (0.18)	< 0.000001[1]
C Peptide (ng/ml)*	9.72 (1.33)	7.39 (2.09)	0.002022[1]	4.19 (1.70)	< 0.000001[1]
Leptin (ng/ml)*	8.45 (6.39)	2.62 (1.29)	< 0.000001[1]	2.65 (0.79)	<0.000001[1]
Adiponectin (μg/ml)*	2.09 (1.09)	3.63 (5.58)	< 0.000001[1]	7.53 (7.42)	<0.000001[1]
GLP-1 (ng/ml)*	47.40 (12.17)	16.12 (17.61)	< 0.000001[1]	- -	-
Ghrelin (pg/ml)*	117.40 (42.9)	94.42 (15.72)	0.001038[1]	84.82 (32.86)	0.000526[1]

*median and IQR (in brackets); [1]Wilcoxon signed rank test; [2]paired T test

Table 6. Comparison of pro-inflammatory markers levels after 12 months of treatment for both groups

Proinflammatory Markers	CTG		P	LSG		P
	V1	V3		V1	V3	
	n=21	n=15		n=20	n=19	
IL-6*	5.79 (13.41)	11.51 (9.04)	0.08325	3.34 (6.26)	11.51 (11.47)	0.0004
TNFα*	0.73 (1.12)	3.27 (3.56)	0.00116	0.65 (0.90)	2.90 (3.33)	0.003342
Homocysteine*	1.94 (0.95)	1.94 (1.53)	0.0637[1]	1.96 (0.88)	2.36 (0.71)	0.1447[1]
hsCRP*	6.34 (6.21)	12.33 (9.35)	0.0946[1]	9.47 (4.85)	1.31 (2.48)	0.0006447[1]

*median and IQR (in brackets); [1]Wilcoxon signed rank test; [2]paired T test

Table 7. Oxidative stress markers in diabetic patients at baseline and at 6 months follow-up

Oxidative Stress Markers	Baseline		6 Months		p value
	CTG	LSG	CTG	LSG	
	n=21	n=20	n=17	n=19	
RB LM/PMA (Maximum RLU)	0.06 ± 0.01	0.19 ± 0.05	0.09 ± 0.02	0.17 ± 0.04	ns
RB LG/PMA (Maximum RLU)	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	0.01 ± 0.00	ns
RB LM/OZ (Maximum RLU)	0.12 ± 0.03	0.33 ± 0.09	0.23 ± 0.06*	0.37 ± 0.00	< 0.05
RB LG/OZ (Maximum RLU)	0.01 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	0.02 ± 0.00	ns
GSH (µg/g Hb)	0.27 ± 0.01	0.26 ± 0.01	0.28 ± 0.01	0.23 ± 0.00**	< 0.05
8-OH-2dG (ng/ml)	10.31 ± 0.54	13.10 ± 2.14	10.15 ± 1.82	12.04 ± 0.70	ns

RB, respiratory burst; PMA, phorbol 12-myristate 13-acetate; ZO, opsonized zymosan; LM, luminol; LG, lucigenin; GSH, glutathione, 8-OH-2dG, 8-hydroxy-2-deoxyguanosine; Maximum, the maximal peak value; RLU, Relative Chemiluminescence Units. Data are expressed as mean ± SEM (standard error of the mean). The p-value refers to the comparison between CTG and LSG after 6 months; * and ** denote $p < 0.05$ and $p < 0.001$ when comparing 6months to baseline within each group; ns = not significant

there is a slight increase at 6 months followed by a higher one at 12 months, denoting the failure of the medical-nutritional intervention in achieving and maintaining the weight loss. The level of homocysteine remained unchanged in both the surgical and conventional groups, indicating that this biomarker is useless in assessing candidates for bariatric surgery.

Analysis of the oxidative stress markers including respiratory burst and levels of glutathione pointed favorable outcomes for the surgical group, as compared with the dietary and intensive medical therapy group (Table 7).

Discussion

The combination between the etiopathogenic mechanisms of T2DM and other factors, such as the excess and distribution of white adipose tissue, its pro-inflammatory state and oxidative/antioxidative imbalance is particular for each clinical situation and explains the metabolic diversity of T2DM [17-19].

LSG has been proposed as an optimal surgical intervention for patients with mild, nonmorbid obesity [20-24]. The mechanism for T2DM remission after LSG is not fully elucidated, involving not only a quick improvement in glucose homeostasis, but also a reduction of other co-morbidities like dyslipidemia, cardiovascular risk factors, hypertension, and obstructive sleep apnea syndrome (OSAS) [24-26]. The results reported in the literature are confirmed by the present study, showing a statistically significant decrease in glycated haemoglobin (HbA1c) level in 89% of the patients from the LSG group compared to only 14% of patients from the conventional treatment group [27]. Beneficial results in the surgical group, as compared with the group receiving intensive medical therapy and diet, were recorded for blood glucose levels, BMI, weight, visceral fat level, HDL-cholesterol and incretin hormones. Also, we have noticed a decrease of pro-inflammatory and oxidative stress markers.

As expected, insulin resistance (evaluated by HOMA-IR) was not significantly improved in the control group (from 8.12 to 6.32, p=0.168), in comparison with the surgical group (from 8.44 to 1.19, p <0.001). Results are comparable to those reported by Schmatz R et al., with mean HOMA-IR decreasing from 6.08 to 1.28 following bariatric surgery in a group of 20 obese T2DM subjects [28].

The results of our study show that diabesity is positively correlated with the intensity of oxidative stress and pro-inflammatory status. Obesity associated with T2DM enhances the pro-inflammatory status due to some specific features of the adipose tissue, like hypertrophy, hyperplasia, a peculiar fat distribution, predominantly abdominal (perivisceral), an increased secretion of adipokines and infiltration of with monocyte-macrophages inducing overloading of the endoplasmic reticulum (ER), which leads to ER dysfunction or ER stress, accumulation of incorrectly folded proteins, adipocyte apoptosis amplifying the inflammatory cascade [29]. An important weight loss can attenuate the pro-inflammatory response of "aggressive" adipocytes regressing to their natural state of "quiet" adipocytes [30]. Antioxidant enzymes GPx and CAT increased after 6 months in both groups.

At 6 months post-surgery, activities of SOD and PON1 (PON1phe and PON1dhc) were not different in the LSG group when compared with CTG group.

Analysis of oxidative stress markers, including RB, 8-OH-2dG, and levels of antioxidant enzymes, after 6 months has also shown favorable results in the surgical groups, as compared with the group receiving intensive medical therapy alone.

Of the investigated parameters, PON1dhc activity correlated positively with concentrations of HDL-C and adiponectin ($p < 0.05$), and negatively with BMI, waist circumference, SBP, levels of HbA1C, insulin and HOMA-IR ($p < 0.05$). The positive correlation between adiponectin and PON1 dhc remained significant even after adjustments for age, gender, BMI, blood pressure, HOMA-IR, HDL-C and LDL-C.

The statistically significant decrease of fat mass and of visceral fat level leads to a decrease in the secretion of leptin and an increase in adiponectin levels, resulting in insulin sensitivity. In the surgical group, leptin decreased significantly ($p<0.001$). A less pronounced but also significant ($p=0.002$) decrease of leptin was recorded in the conventional group, so that finally the change at one year was not significant between the two groups ($p=0.51$). The decrease of leptin levels in LSG patients was previously reported [31,32] and is explained by the decrease of fat tissue mass in parallel with weight loss. The levels of adiponectin were correlated negatively with the waist circumference ($r = -0.49$, $p < 0.001$), diastolic BP ($r = -0.30$, $p < 0.05$), levels of uric acid ($r = -0.33$, $p < 0.001$), glucose ($r = - 0.30$, $p < 0.05$).

Overall, the percentage of excess weight loss (EWL) in the LSG group was 78.98% in comparison with only 9.46% in the control group. An interesting observation regarding the levels of pro-inflammatory markers is that for both studied groups at V2 there is an increase in circulating levels of IL-6 and TNF- α followed by a decrease with a stabilization at V3, after 12 months despite the continuing loss of adipose mass recorded in the eugical group. Tracking patients in follow-up for more than 1 year would have brought more information about the trend of decrease / stabilization of the cytokine's levels. The levels of C reactive protein (hsCRP), an acute phase reactant protein involved in the early stages of the inflammatory process has shown a cintinuous decreasing trend in time. We recorded a strong decrease for LSG group ($p<0.001$) and a mild decrease for the conventional group ($p=0.09$).

The reduced caloric intake immediately after surgery is causing the decrease of adipose tissue mass, changes in the incretin hormonal status and in intestinal absorption, achieving the remission of diabetes associated with obesity [24-26].

Regarding the incretins, ghrelin and the glucose dependent insulinotropic polypeptide GLP-1, are the most likely candidates for increasing insulin sensitivity after this type of surgery, even before the occurrence of substantial weight loss. GLP-1 is secreted by endocrine L-cells in the mucosa of the ileum and colon. Considerable attention has focused on GLP-1 which regulates glucose homeostasis in T2DM patients, modulates gastric emptying and acid secretion and also exhibits dual actions in glucose homeostasis through its concurrent insulinotropic and glucagonostatic actions [33]. In addition, ghrelin levels are reduced due to the LSG resection of gastric fundus cells [34]. Decreasing the gastric volume has a direct consequence not only on lowering the level of ghrelin, but also leads to decreased appetite and food intake [23].

Ghrelin is a peptide produced by the fundus and body of the stomach and duodenum, being named after its role as a growth hormone-releasing peptide (GHRe-lin) [35]. It is the only known orexigenic gut hormone. Additional evidence suggests that ghrelin may also contribute to long-term body weight regulation, being a potential therapeutic candidate in the fight against obesity. Ghrelin contributes to preprandial hunger and meal initiation [36-43].

Mainly seen as an indicator for impaired β-cell function, proinsulin can be detected at low concentrations in the blood of healthy persons but is found at higher concentrations in the blood of insulin-resistant subjects [36] and patients with T2DM [37-39]. There were no

statistically significant correlations between ghrelin and all of the studied parameters.

As expected, patients in the surgical group harbored a significant decrease of intact proinsulin (from 5.86 to 0.70 pmol/L, $p<0.001$), indicating improvements of beta cell dysfunction or a beta cell "rest state" after metabolic surgery [44]. We have also found a significant decrease of intact proinsulin in the control group (from 6.42 to 3.06 pmol/L, $p=0.0011$) despite the modest weight loss and metabolic improvement in these patients.

This might suggest that lifestyle changes, even when they are not accompanied by significant weight loss, may have a positive impact on beta cell function. The efficiency of β-cells to convert proinsulin to insulin depends on endoplasmic reticulum (ER) packaging or folding capacity, the available space for protein folding, the clearance rate of misfolded proteins to avoid accumulation of toxic debris and last but not least, the RE ability to efficiently carry the folded proinsulin to the next secretory compartment (Golgi apparatus) [45]. Therefore, high levels of proinsulin indicate an advanced stage of depletion of pancreatic beta cells and represent a high specificity marker for insulin resistance. It can be used as an arbitrary marker to determine the therapeutic decision in T2DM. Thus, the evaluation of beta cell secretion should include, besides insulin and C peptide serum levels; proinsulin, that provides valuable information on the location of the insulin-secreting defect and the progressive or regressive course of the pathogenic process in T2DM. Similar changes were found for the proinsulin-to-insulin ratio, also a valuable indicator of beta cell dysfunction [44,46-48]. The proinsulin to insulin ratio decreased for LSG subjects ($p<0.001$), demonstrating that the secretory burden of the pancreas decreases, and the secretory response improves. This ratio shows the relationship between the secretory demand and the secretory response of the pancreas. Debates still exist on which factors play an important role in predicting the outcome of bariatric-metabolic surgery on T2DM remission. It is well known that ABCD score includes age, BMI, C peptide and duration of the disease. Other predictive score of T2DM remission is DiaRem which includes age, HbA1c, antidiabetic drugs and insulin [49].

Lower values of HDLc are also correlated with a lower probability of T2DM remission post-surgery. Highly sensitive C reactive protein (hsCRP) is an indicator of the non-specific systemic inflammatory process associated with T2DM, which in turn correlates with pancreatic β-cell apoptosis and decreased insulin secretion reserve. High values of hsCRP correlate with a low insulin secretory reserve and therefore, a less probability of post-surgical T2DM remission. Adiponectin is an indirect indicator of insulin resistance, a low adiponectin level suggesting a higher degree of insulin resistance. In our study, lower preoperative values (higher insulin resistance) have been associated with a lower probability of remission of T2DM after metabolic surgery.

Conclusions

LSG appears to be a promising alternative for T2DM therapy, even in non-morbidly obese patients. In addition, proinsulin levels, proinsulin / insulin ratio and the visceral fat percentage can be considered valuable markers for predicting the progressive / regressive trend of pathogenic processes linked to diabetes. The limitations of this study are the small number of patients enrolled in the study and the short duration of the monitoring period. However, these limitations are offset by the large panel of biomarkers surveilled over one year that could offer an overview of the metabolic, inflammatory and oxidative stress profile of T2DM obese patients undergoing different therapeutic interventions.

Acknowledgments

This work was funded through the Programme "Partnership in priority areas" by the UEFISCDI, project number PN-II-PT-PCCA-2013-4-2154. We are grateful to acknowledge Janeta Tudosoiu from the research laboratory of NIDNMB "Prof.N.C.Paulescu", for excellent technical assistance and our statistician, Teddy Popescu from Ponderas Academic Hospital.

Conflicts of Interest

The authors have declared that there is no conflict of interest.

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