Comparison of Sleeve Gastrectomy and Conservatory Treatment Effect on Biochemical and Hormonal Profile of Obese Type 2 Diabetes Subjects: CREDOR Randomized Controlled Study Results

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Metabolic surgery is the most efficacious method for the treatment of morbid obesity and was recently included among the antidiabetes treatments recommended in obese type 2 diabetes (T2D) patients. The aim of this study was to compare in a randomized controlled trial the effect of sleeve gastrectomy (SG) to that of intensive lifestyle intervention plus pharmacologic treatment on some markers of insulin resistance and beta cell function as well as some appetite controlling hormones in a group of male obese T2D subjects. The study groups comprised 20 subjects for SG and 21 control subjects. Fasting blood glucose, insulin, proinsulin, adiponectin, leptin, ghrelin, HOMA-IR, HOMA-%B, proinsulin-to-insulin ratio and proinsulin-to-adiponectin ratio were evaluated at baseline and after one year follow-up. Overall, patients in the SG group lost 78.98% of excess weight loss (%EWL) in comparison with 9.45% in the control group. This was accompanied by a significant improvement of insulin resistance markers, including increase of adiponectin and decrease of HOMA-IR, while no changes were recorded in the control group. Weight loss was also associated with a significant improvement of proinsulin-to-insulin and proinsulin-to-adiponectin ratio, both surrogate markers of beta cell dysfunction. These also improved in the control group, but were only marginally significant. Our findings suggest that improved insulin resistance and decreased beta cell dysfunction after sleeve gastrectomy might explain diabetes remission associated with metabolic surgery.

Keywords: hormones, obesity, diabetes, weight loss, lifestyle intervention, sleeve gastrectomy

Diabetes is one of the most frequent non-communicable diseases worldwide, with mean prevalence rates around 9% and an estimated 415-422 millions of patients affected worldwide in 2014/2015 [1,2]. It is expected that this figures will rise to 10.5% prevalence and 642 million patients by 2040 [1]. In the same time, it is one of the most important causes of mortality, morbidity and health-care related costs [2]. One of the main recognized drivers of the epidemics of type 2 diabetes (T2DM) is represented by obesity [3]. Obesity, another progressive chronic disease determined by genetic, environmental but also behavioural factors, has also reached epidemic proportions all over the world, with more than 600 million obese subjects worldwide in 2014 [4], with an alarming increase in prevalence in children and adolescents [5]. Obesity and its health related complications (including T2DM) have a great impact on the patient (both physically and psychologically) and huge social costs [6].

The cornerstone of therapeutic interventions to prevent/treat obesity and T2DM is weight loss and the first recommendation is for lifestyle change, including reduced caloric intake (diet) and increased energy expenditure (exercise) [7]. Typical weight loss resulting from lifestyle change is 5–10% of baseline weight, but even modest weigh loss (5% of body weight) might improve metabolic parameters [8], so lifestyle intervention is first line therapy for T2DM. Unfortunately, quite often initial success of lifestyle intervention in weight loss is followed by weight regain [9], so that lifestyle alone is successful only in a small number of T2DM subjects [3].

Due to the progressive nature of T2DM, with continuous decline of the beta cell function, pharmacological treatment is recommended from diagnosis (usually with metformin) and most often needs to be periodically intensified, in order to maintain proposed targets [8]. Finally insulin treatment is initiated and, subsequently, intensified to multiple shots daily, with the characteristic side effects of weight gain and hypoglycemia. Despite the current availability of at least 11-12 different of anti-diabetes drug classes [10], the majority of T2DM patients fail to reach or maintain good metabolic control [11], exposing themselves to the risk of invalidating chronic complications, cardiovascular events and, finally, decreased life expectancy.

Bariatric surgery interventions were first introduced in the 1950's to promote weight loss and meanwhile have become the most successful procedures to induce substantial and long lasting weight loss for morbidly (body mass index BMI > 40 kg/m^2) obese subjects [12]. By mechanically changing the normal physiology of gastro-intestinal kinetics and nutrient absorption, bariatric surgery is associated with a spectacular weight loss as well as positive effects on diabetes metabolic control, diabetes outcomes, cardiovascular events and, finally, decreased mortality in these subjects [13]. In the same time, bariatric

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surgery proved to be the most efficient method for the treatment of T2DM. In 1995, Pories et al. published a famous article entitled Who Would Have Thought It? An Operation Proves to Be the Most Effective Therapy for Adult-Onset Diabetes Mellitus and reported post-surgical remission of T2DM in up to 78% of patients [14]. Subsequently, a large body of evidence accumulated indicating long term improvement rates of T2DM that correlate with the extent of weight loss, being higher after malabsorbtive/mixed procedures (Roux-en-Y gastric bypass (RYGP) and biliopancreatic diversion with up to 80-90% improvement of diabetes at 2 years) and lower after pure restrictive procedures (sleeve gastrectomy and adjustable gastric banding with up to 50-60% improvement of diabetes at 2 years) [15]. All these led to the new designation of these interventions as metabolic surgery and their inclusion amongst the methods for the management of T2DM [16]. According to these, metabolic surgery should be recommended to treat T2DM in patients with class III obesity (BMI > 40 kg/m^2) and in those with class II obesity (BMI 35.0–39.9 kg/m^2) when hyperglycemia is inadequately controlled by lifestyle and optimal medical therapy. Surgery should also be considered for patients with T2D and BMI 30.0–34.9 kg/m^2 if hyperglycemia is inadequately controlled despite optimal treatment with either oral or injectable medications.

There are several hypotheses regarding the mechanism of diabetes improvement after metabolic surgery [17], starting from the obvious effects of decreased caloric intake and weight loss on insulin sensitivity, to the role of the gastrointestinal tract hormones (including glucagon-like peptide 1 – GLP-1, glucose-dependent insulinotropic polypeptide – GIP, ghrelin, oxyntomodulin, peptide YY, etc.), changes in bile acids metabolism and the profound changes of the intestinal microbiome following surgery.

Obesity and type 2 diabetes represent major health care issues also in Romania. Results of the recent PREDATORR epidemiological study estimated the prevalence of diabetes at 11.3% of adult population and that of overweight and obesity at 66% [18]. Metabolic surgery procedures are performed in Romania, mainly in excellence centers [19] but, to our best knowledge, their effect was not compared with that of medical treatment in obese T2DM subjects in a prospective randomized study. The CREDOR (Collaborative Romanian Efforts for Diabetes and Obesity Retrench) study was initiated in 2014 with the aim to compare the results of metabolic surgery (gastric sleeve) with those of conservatory treatment (lifestyle changes and medication) on T2DM remission after one year of follow-up. It was a prospective, randomized controlled trial with an overall duration of 12 months and clinical/biological evaluations of study subjects at baseline, 6 and 12 months.

The aim of this study was to compare the effects of sleeve gastrectomy to that of standard conservatory medical treatment on some markers of insulin resistance and beta cell dysfunction as well as on some appetite-controlling hormones in the CREDOR trial subjects.

Experimental part

Study groups

Forty one type 2 diabetes males with known obesity (BMI above 30 kg/m^2) were selected for the CREDOR study in the period September to December 2014. Inclusion and exclusion criteria were previously described [20,21]. Briefly, key inclusion criteria were: age between 30-65 years, diabetes duration 1-15 years, acceptance and financial capability to cover the costs of nutritional supplements after sleeve gastrectomy intervention. Key exclusion criteria were: type 1 diabetes, HbA1c < 6.5%, fasting C-peptide < 0.81 ng/mL, Hemoglobin < 10 g/dL, serum creatinine > 1.2 mg/dL or GFR < 60mL/min/1.73 m^2, NYHA III/IV heart failure, respiratory failure, acute CV events (myocardial infarction or stroke) in the previous year, coronary, carotid or peripheral arteries revascularization during the previous 6 to 12 months, cirrhosis, liver failure, any chronic pathology of the digestive system, positive serology for B or C hepatitis or HIV.

Approval from the ethics committees of each participating trial center was obtained prior to the study. Written informed consent was obtained from all patients prior to their inclusion in the study and the trial was conducted in accordance with the Declaration of Helsinki.

Randomization and interventions

Patients were randomized in 2 groups: Group 1 (Control) - conservatory treatment of diabetes and obesity (n=21), and Group 2 (Sleeve Gastrectomy) – surgical laparoscopic sleeve gastrectomy (n=20). All patients were fully evaluated at baseline (Visit V1) when all patients from the conservatory group they were given a personalized nutritional intervention program. The calorie intake for each subject was calculated starting from the estimated Resting Metabolic Rate (RMR) according to the formula: [RMR*1.3 (sedentary lifestyle) + (10% * RMR) – 500], thus inducing a 500 kcal daily restriction. In addition, all subjects received lifestyle counseling regarding the increase of physical activity (light or moderate exercise at least 30 min, 3 to 5 times per week), smoking cessation and alcohol intake. Multifactorial treatment of diabetes, hypertension and dyslipidemia according to current guidelines was provided. Patients from the surgical group underwent a sleeve-gastrectomy laparoscopic procedure. Subsequently, they received specific dietary advice (vitamin and minerals supplementation) and down-titration of diabetes medication (if necessary) according to their blood glucose profiles. Patient disposition is given in figure 1. The reasons for missing follow-up visits were patient's decision to withdraw from the study for the conservatory group, while 1 patient was lost to follow-up in the surgical group.

Anthropometric measurements

At each study visit, body weight was measured using a Tanita BC-418MA Body Composition Analyzer and blood samples were drawn and samples of serum and plasma were preserved for future biological determinations. Weight loss at follow-up visits was assessed using the Percentage Excess Weight Loss (%EWL) formula: (Initial weight – Final weight)/(Initial weight – Ideal weight) x100 [22], whereas ideal weight was calculated using the Metropolitan Life Insurance formula.

Height was measured using a height measuring tape while the waist circumference (WC) was measured with a standard measuring tape.

Biological measurements

Venous blood was collected after an overnight fasting , in vacuum tubes without anticoagulant (for biochemistry tests, HIV, HBV, HCV and ELISA assays), vacuum tubes with EDTA (for hemoglobin and HbA1c assay) and vacuum tubes with aprotinin (for ghrelin assay). Hemolyzed, lipemic and intense opalescent blood samples were considered non-compliant and were excluded.

Fasting serum glucose (separated after a 10 min centrifugation at 2200 rpm) was measured by spectrophotometric methods using an EOS BRAVO FORTE

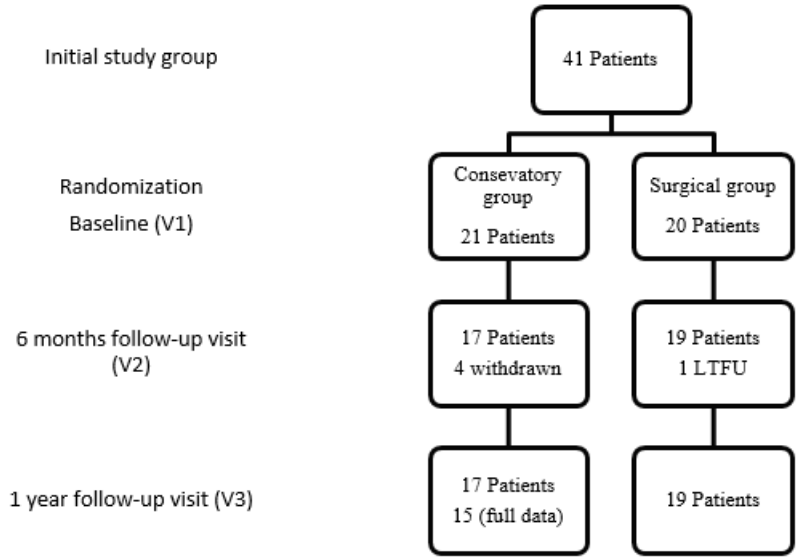

Fig. 1. Patient disposition

HOSPITEX DIAGNOSTICS biochemistry analyzer, with the specific reagents recommended by the producer.

The fresh whole blood samples with EDTA were used to determine glycated hemoglobin (HbA1c) - by HPLC (high performance liquid cromatography method) on D10-Biorad analyzer and hemoglobin – by automatic photometric method on Cell-Dyn 3700 Biorad analyzer.

Immunological determinations were performed on thawed serum and plasma samples which were previously separated, aliquoted, labelled and stored at -80° C. Serum proinsulin, insulin, leptin, adiponectin levels and plasma ghrelin level were measured by ELISA (Enzyme-Linked Immunosorbent Assay) method using commercially available kits from DRG Instruments GmbH, Germany (proinsulin - EIA-1560 DRG, coefficient of variation (CV) = 4.86, sensitivity < 0.5 pmol/L; insulin - EIA-2935 DRG, CV = 2.2, sensitivity = 1.76 μIU/mL; leptin - EIA-2395 DRG, CV = 6.43, sensitivity =1 ng/mL); adiponectin - EIA-4177 DRG, CV = 3.36, sensitivity = 0.2 ng/mL; ghrelin - EIA-4710 DRG, CV = 2.31, sensitivity = 15 pg/mL) according to the producer recommendations. Absorbances were read at 450 nm using an automatic ELISA plate reader: *MULTISKAN Ex-Thermo Electro Corporation* (CV=2.6). Proinsulin-to-insulin and proinsulin-to-adiponectin ratios were calculated.

Based on fasting blood glucose and fasting insulin levels, insulin resistance was estimated using the Homeostasis Model Assessment for Insulin Resistance (HOMA-IR) formula: [(fasting blood glucose (mmol/L) x fasting insulin (mU/L))/22.5]. In addition, beta cell function was estimated according with the Homeostasis Model Assessment for â cell function (HOMA%B) formula: (20 x fasting insulin (mU/L)/(fasting blood glucose (mmol/L)-3.5) [23]. We chose to use fasting insulin in the calculations (despite the fact that several patients were treated with exogenous insulin) since there are data indicating the validity of this method even in insulin treated T2DM patients [24].

Statistical analysis

All statistical analyses were conducted using the SPSS® version 20.0 software. We used mean ± standard deviation to describe continuous variables with a normal distribution and median with interquartile range (in brackets) for variables with skewed distribution. Paired Student's *t*-tests and Wilcoxon signed rank test were used as appropriate to compare data from the 2 groups. A p value <0.05 was considered statistically significant.

Results and discussions

The demographic and clinical characteristics of the subjects at baseline were similar between the two treatment groups as shown in Table 1. Overall patients were relatively young, with a mean BMI > 40 kg/m^2 (indicating morbid obesity) and poor metabolic control (mean HbA1c higher than 8%) despite relatively short duration of T2DM.

The results of sleeve gastrectomy vs. conservatory treatment at one year on clinical parameters including weight, BMI, diabetes metabolic control, blood pressure and lipid parameters will be reported elsewhere (Smeu B et al. unpublished data). Briefly, patients in the sleeve gastrectomy group decreased significantly their BMI and weight in comparison with patients from the control group. Overall, the percentage of excess loss (EWL) in the sleeve gastrectomy group was 78.98% in comparison with only 9.46% in the control group.

Insulin resistance (evaluated by HOMA-IR) improved non-significantly in the control group (from 8.1 to 6.3, $p=0.17$) and significantly in the surgical group (from 8.4 to 1.2, $p<10^{-6}$), the difference between the groups being significant, with $p=0.04$ (table 2). Results are comparable with those reported by Schmatz R et al., with mean HOMA-IR decrease from 6.08 to 1.28 following bariatric surgery in

	Control (n = 21)	Sleeve gastrectomy (n = 20)
Mean age (years)	48.7±6.8	46±5.9
Diabetes duration (years)	6.3±4.5	5.4±2.9
BMI (kg/m^2)	41.51±5.56	41.2±4.8
Waist circumference (cm)	135.9±10.72	139.85±16.69
HbA1c (%)	8.35±1.49	8.82±1.56

Table 1
Baseline characteristics of patients

Table 2

ONE YEAR CHANGE IN INSULIN RESISTANCE AND BETA CELL FUNCTION MARKERS IN SLEEVE GASTRECTOMY VS. CONTROL PATIENTS

Parameter	Control (n=15)			Sleeve Gastrectomy (n=19)			SG vs. Control at one year
	Baseline	One year	p*	Baseline	One year	p*	p*
Insulin (μIU/mL)	22.1 (23.4)	14.1 (31.8)	0.2	18.9 (24.4)	5.1 (4.2)	$<10^{-6}$	0.008
Proinsulin (pmol/L)	6.4 (19.8)	3.1 (5.3)	0.001	5.9 (6.6)	0.7 (0.9)	$<10^{-6}$	0.45
Adiponectin (μg/mL)	4.8 (9.3)	3.5 (2.6)	0.08	2.1 (1.1)	7.5 (7.4)	$<10^{-6}$	$<10^{-6}$
Proinsulin-to-insulin ratio	0.4 (0.6)	0.2 (0.2)	0.09	0.3 (0.3)	0.1 (0.2)	$<10^{-6}$	0.60
Proinsulin-to-adiponectin ratio	1.8 (1.9)	1 (1.6)	0.02	2.5 (3.7)	0.1 (0.2)	$<10^{-6}$	0.06
HOMA-IR	8.1 (14.0)	6.3 (11.9)	0.17	8.4 (10.6)	1.2 (0.8)	$<10^{-6}$	0.04
HOMA-%B	90.7 (52.2)	67.5 (146.8)	0.76	60.2 (84.7)	61.3 (49.3)	0.54	0.39

All data are expressed as median and (interquartile range); * Wilcoxon signed rank test

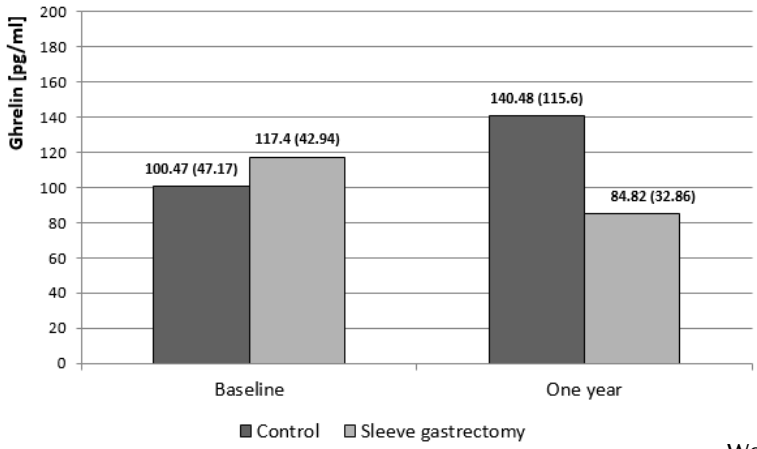

Fig. 2. One year changes in ghrelin levels in sleeve gastrectomy vs. control patients

a group of 20 obese T2DM subjects [25]. Change of HOMA-IR was concordant with the significant increase of adiponectin levels in the surgical group (table 2), while change was minor in the control group, the difference between groups being highly significant ($p < 10^{-6}$). Again, this was similar with previously published data indicating marked increases of this adipokine following bariatric procedures [26].

We used proinsulin as a marker for beta cell dysfunction, as suggested by previous reports [27-29]. As expected, patients in the surgical group had a significant decrease of intact proinsulin (from 5.9 to 0.7 pmol/L, $p < 10^{-6}$), indicating improvement of beta cell dysfunction after metabolic surgery, a phenomenon described from the first cohorts of baritatric patients [30]. We also found a significant decrease of intact proinsulin in the control group (from 6.4 to 3.1 pmol/L, p=0.001) despite the modest weight loss and metabolic improvement in these patients. This might suggest that the change in lifestyle, even when not accompanied by important weight loss, may have a positive impact on beta cell function.

Similar patterns of change were found for the proinsulin-to-insulin and proinsulin-to-adiponectin ratio, both surrogate indicators of beta cell dysfunction [28,31], but overall, change at one year was not different between the two study groups. Detailed values of the studied parameters of insulin resistance and beta cell function are provided in table 2.

We also analyzed the effect of sleeve gastrectomy on the levels of some appetite-controlling hormones, including leptin and ghrelin. Thus, we found a statistically significant decrease of ghrelin levels in sleeve gastrectomy patients, from 117.4 pg/mL to 84.82 pg/mL, p=0.0005 (fig. 2). In the same time, ghrelin increased non-significantly in the control group, from 100.47 pg/ml to 140.48 pg/mL, p=0.09. Overall, the difference between the two study groups at one year was significant, with p=0.0016. The decreased level of ghrelin in sleeve gastrectomy patients was expected, being previously reported [32,33] and explained by the reduction of ghrelin secreting cells following resection of the gastric fundus during the surgical procedure [34]. The increased ghrelin levels in the control group might explain weight regain that occurred between 6 months and 12 months in these patients (data not shown). This could represent a phenomenon of metabolic adaptation to weight loss [35,36], resulting in increased hunger, caloric intake and limitation of weight loss.

The evolution of leptin levels in the two study groups is given in figure 3. In the surgical group, leptin decreased significantly ($p < 10^{-6}$). A less pronounced but also significant (p=0.003) decrease of leptin was recorded in the control 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 sleeve gastrectomy patients was previously reported [33,37,38, 41] and is explained by the decrease of fat tissue mass in parallel with weight loss

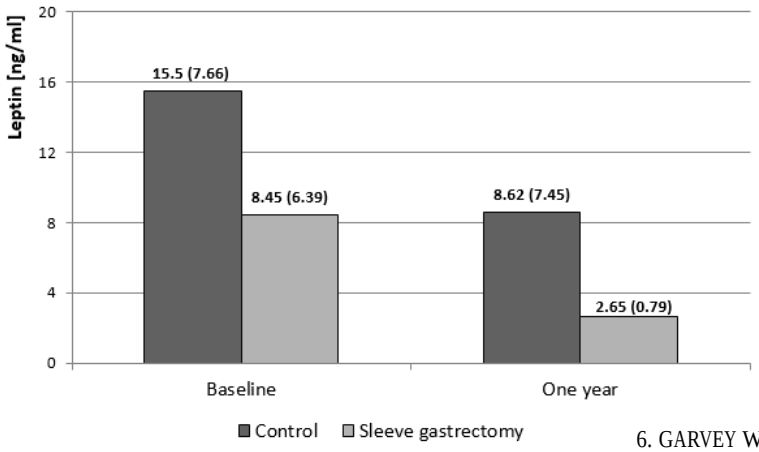

Fig. 3. One year changes in leptin levels in sleeve gastrectomy vs. control patients

(in our study percentage of fat mass decreased from 32.7% to 20.3% in the surgical group). For the control group, the decrease in leptin levels was disproportionate when compared with the modest weight loss. This is again a phenomenon previously described that might limit the efficacy of lifestyle interventions on the long term [35, 39,40].

Our study has several limitations. First, the number of subjects included in the study was quite low and the follow-up period was limited to one year (both due to limited funding). Second, the study included only male subjects (as a consequence of the low number of patients, this was decided in order to increase the homogeneity of the study group). This precludes generalization of data to the whole population of obese subjects with T2DM. Expansion of the study group and longer follow-up will be required before drawing definite conclusions. Third, we had a quite large percentage of drop-outs from the control group, explained by the difficulties to adhere on the long term to lifestyle changes. However, the study has also some strengths, including its prospective design. Most importantly, this was the first randomized controlled trial in Romania comparing the efficacy and safety of sleeve gastrectomy with that of standard medical intervention in a population of obese T2DM subjects.

Conclusions

As expected, sleeve gastrectomy led to significant weight loss in comparison with the conservatory approach. This was associated with improvement of insulin resistance and decrease of beta cell dysfunction, possibly explaining the "diabetes remission" associated with metabolic surgery. The increase in ghrelin and decrease of leptin levels in subjects receiving lifestyle counselling on top of standard medical treatment might explain its reduced efficacy in inducing weight loss.

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