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Beyond Restrictive: Sleeve Gastrectomy to Single Anastomosis Duodeno-Ileal Bypass with Sleeve Gastrectomy as a Spectrum of One Single Procedure

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Keywords

Restriction · Hypoabsorptive procedure · Two-stage surgery · Weight loss mechanisms

Abstract

Introduction: Single anastomosis duodeno-ileal bypass with sleeve gastrectomy (SADI-S) is a restrictive/hypoabsorptive procedure recommended for patients with obesity class 3. For safety reasons, SADI-S can be split into a two-step procedure by performing a sleeve gastrectomy (SG) first. This stepwise approach also provides an unprecedented opportunity to disentangle the weight loss mechanisms triggered by each component. The objective was to compare weight trajectories and post-prandial endocrine and metabolic responses of patients with obesity class 3 submitted to SADI-S or SG as the first step of SADI-S. ***Methods:*** Subjects submitted to SADI-S ($n = 7$) or SG ($n = 7$) at a tertiary referral public academic hospital underwent anthropometric evaluation and a liquid mixed meal tolerance test (MMTT) pre-operatively and at 3, 6, and 12 months post-operatively. ***Results:*** Anthropometric parameters, as well as metabolic and micronutrient profiles, were not significantly different between groups, neither before nor after surgery. There were no significant differences in fasting or post-prandial glucose, insulin, C-peptide, ghrelin, insulin secretion rate, and insulin clearance during the MMTT between subjects submitted to SADI-S and SG. There was no lost to follow-up. ***Conclusions:*** The restrictive component seems to be the main driver for weight loss and metabolic adaptations observed during the first 12 months after SADI-S, given that the weight trajectories and metabolic profiles do not differ from SG. These data provide support for surgeons' choice of a two-step SADI-S without jeopardizing the weight loss outcomes.

Introduction

Biliopancreatic diversion with duodenal switch (BPD/DS) is the most effective weight loss intervention for the treatment of patients with obesity class 3 [1, 2]. Single anastomosis duodeno-ileal bypass with sleeve gastrectomy

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(SADI-S) is another restrictive/hypoabsorptive bariatric procedure that has been replacing BPD/DS, being technically simpler with comparable short-term results [3]. The fact that both procedures, BPD/DS and SADI-S, are feasible in a two-step approach with the restrictive component first, increased the popularity of sleeve gastrectomy (SG) in patients with severe obesity, not only as a first step, but also as a standalone technique [4].

Weight loss achieved in the first 12 months after bariatric surgery is known to be a major determinant of primary response and a prognostic factor for long-term outcomes [5]. Notwithstanding, prospective randomized trials comparing outcomes of one and two-step BPD/DS or SADI-S, with well-defined intervals between surgeries, are lacking. Understanding how does weight loss achieved by SG as a first step compared to a single-step intervention will allow to evaluate the impact of a two-step BPD/DS or SADI-S approach on surgical outcomes.

Bariatric procedures, including BPD-DS and SADI-S, modify post-prandial hormonal and metabolite profiles, contributing to weight loss through food intake restriction and hypoabsorption [6–9]. To what extent these molecular adaptations are modified when surgeries are performed in two steps is unknown. Indeed, SG was also shown to induce hormonal and metabolic adaptations [10], including improved glucose tolerance, insulin sensitivity, pancreatic β-cell function, and increased post-glucose incretin hormone response [11–13]. How does the gastrointestinal adaptation between surgeries influences the outcomes of a second intervention remains to be demonstrated. Understanding the mechanisms underlying weight loss induced by SG as a first step would provide a rationale for deciding between one- or two-step intervention strategies. Moreover, decomposing SADI-S into its components of restriction and hypoabsorption represents an unprecedented opportunity for characterizing the molecular mechanisms underlying weight loss elicited by each step.

As we delved into the possibility of SG and SADI-S being a spectrum of one single surgical procedure, we became aware that this hypothesis remained to be tested. The primary goal of this study was to assess weight trajectories and endocrine/metabolic post-prandial responses of patients with obesity class 3 submitted to SADI-S or SG, up to 12 months post-operatively.

Methods

This was a prospective, interventional, open-label randomized study, derived from the Surgical Innovation for Diabetes Treatment 2 (SURIDIAB2), with the ClinicalTrials.gov Identifier: NCT04712409. The study was conducted at a single bariatric public hospital center and was approved by the Institutional Ethical Review Board (CA-110/2020-0t_MP/AC). All participants signed written informed consent forms prior to any study intervention. We used the CONSORT checklist when writing our report [14].

Study Population

This study enrolled subjects with obesity ($n = 14$) submitted to SADI-S ($n = 7$) or SG as the first step of a two-step SADI-S ($n = 7$). Participants were recruited from referrals to the obesity surgery multidisciplinary team, between January 2020 and December 2021. Male or female adults, up to 65 years old, with a BMI between 45 and 55 kg/m^2, were included. Prior abdominal surgery, including bariatric interventions, was a pre-specified exclusion criterion. Eligible patients, who accepted to participate, were enrolled and randomized into one of the two study arms. A representative flow diagram of patients enrolled in the randomized study is depicted in Figure 1. Patient groups were paired for gender, age, body weight, BMI, and obesity comorbidities (Table 1). Prediabetes, type 2 diabetes, and metabolic syndrome were defined as per international standard guidelines [15–17].

Study Design

Participants were scheduled for study visits before and 3, 6, and 12 months after surgery. Pre- and post-operative assessments included medical history, physical examination including anthropometrics to calculate BMI, total weight loss (TWL), and percentage of excess BMI loss (%EBMIL). In each visit, subjects underwent a mixed meal tolerance test (MMTT) consisting of the ingestion of a commercially available standardized liquid mixed meal (Fresubin Energy Drink, 200 mL, 300 kcal [50 E% carbohydrate, 15 E% protein, and 35 E% fat]), after 12 h overnight fast and over a maximum period of 15 min. Venous blood samples were collected into EDTA tubes (S-Monovette® 7.5 mL, K2 EDTA Gel, 1.6 mg/mL, Sarstedt), before the meal (−30 and 0 min) and at 15, 30, 45, 60, 90, and 120 min after meal initiation. Samples were centrifuged, and the plasma was separated and stored at −20°C until assayed.

Surgical Procedures

SG consisted of a vertical gastric section starting 4 cm proximal to the pylorus, using a 36-French bougie for calibration. For patients undergoing SADI-S, surgery proceeded by ligating the right gastric artery; sectioning the duodenum 2–3 cm distally to the pylorus; and performing a hand-made 3-layer duodenal-ileal end-to-side anastomosis to create the efferent absorptive limb of 300 cm running until the ileocecal valve. Mesenteric defects were closed. All surgeries were performed laparoscopically by the same surgical team.

Biochemical Measurements

Along the MMTT time-points, capillary blood glucose was measured using a glucometer (Freestyle Precision Neo Glucose meter, Abbott, USA), and insulin and C-peptide plasma levels were measured by an electrochemiluminescence sandwich immunoassay on Atellica® (Siemens). Fasting total ghrelin was measured by ELISA (EZGRT-89K, EMD Millipore, Merck KGaA, Darmstadt, German), following the manufacturer's instructions.

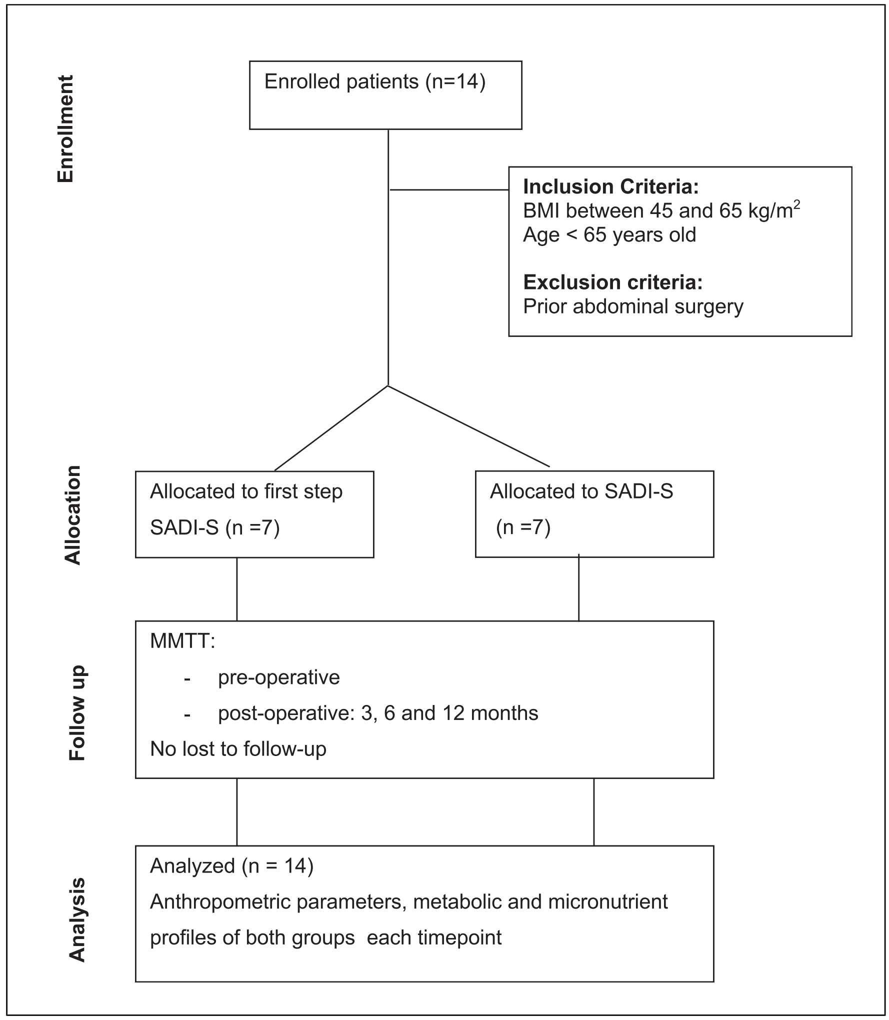

Fig. 1. Flow diagram of patients enrolled in the randomized trial. Fourteen patients were recruited through referrals to the obesity surgery multidisciplinary team and randomly assigned to 2 arms – 7 patients for a one-step SADI-S procedure and 7 patients for a two-step SADI-S procedure. Over the initial year, an MMTT was conducted at 3, 6, and 12 months, evaluating anthropometric parameters and metabolic and nutritional profiles. This assessment aimed to compare the outcomes of SADI-S as the first step in a two-step procedure. BMI, body mass index; SADI-S, single anastomosis duodeno-ileal with sleeve gastrectomy; MMTT, mixed meal tolerance test.

Table 1. Pre-operative features of patients submitted to SADI-S or SG

	SADI-S	SG	*p* value
N (% of total)	7 (50)	7 (50)	–
Sex (female/male)	6/1 (86%/14%)	5/2 (71%/29%)	1.00
Age, years	44±4	40±5	0.74
Body weight, kg	127.6±6.5	125.4±5.0	1.00
BMI, kg/m^2	48.4±0.7	48.1±1.2	1.00
Hyperlipidemia	4 (57%)	4 (57%)	1.00
Hypertension	3 (43%)	3 (43%)	1.00
Prediabetes	3 (43%)	2 (29%)	1.00
T2D	1 (14%)	1 (14%)	1.00
MS	4 (57%)	6 (86%)	0.56

Data are presented as mean ± SEM or number (%), as appropriate. SADI-S single anastomosis duodeno-ileal bypass with sleeve gastrectomy; SG, sleeve gastrectomy; BMI, body mass index; T2D, type 2 diabetes mellitus; MS, metabolic syndrome; SEM, standard error of mean.

Hormonal and glucose total area under the curves (tAUC) were calculated through the trapezoidal rule. Prehepatic insulin secretion rate (ISR) was calculated from C-peptide plasmatic levels, using the ISEC software (ISEC, Version 3.4a, Hovorka, 1994) [18]. Insulin clearance was then obtained according to the formula: ($tAUC_{ISR}/tAUC_{insulin}$).

Statistical Analysis

The samples size was estimated to detect a difference of 10% TWL between the groups, at 12 months, with a power of 85% and an alpha value of 0.05, using the PS Power and Sample Size Calculations software v 3.1.6, for Windows. Based on the coefficient of variation from our preliminary results, we determined that 6 patients would be necessary in each study group.

Nominal variables are expressed as number of cases and percentage (%), and the continuous variables are expressed as mean ± standard error of mean. Normality was evaluated using D'Agostino and Pearson test. Given that no variable passed this test, the comparison of means was obtained through a Mann-Whitney test. A Dunn's test was used to compare BMI, TWL, and %EBMIL changes from pre-operative time, within each of the two groups during follow-up. Comparisons between time-points

Table 2. Anthropometric and clinical data before and after surgery

	Pre-op	3 months	6 months	12 months
BMI, kg/m^2				
SADI-S	48.6±0.9	39.5±0.7	34.1±0.7*	28.2±0.9*
SG	47.1±1.0	36.7±0.9	32.3±1.0*	28.6±0.8*
p	0.83	0.17	0.61	1.00
TWL, %				
SADI-S	–	18.4±1.2	29.5±1.4	41.6±2.1*
SG	–	23.5±1.7	32.5±2.2	40.4±1.7*
p	–	0.14	0.73	0.99
EBMIL, %				
SADI-S	–	38.1±2.5	61.1±2.8	86.1±3.9*
SG	–	49.1±3.4	68.0±4.2	84.6±3.1*
p	–	0.09	0.59	1.00
Hyperlipidemia, *n* (%)				
SADI-S	4 (57)	2 (29)	2 (29)	2 (33)
SG	4 (57)	2 (29)	2 (29)	2 (29)
p	1.00	1.00	1.00	1.00
Hypertension, *n* (%)				
SADI-S	3 (43)	0 (0)	0 (0)	0 (0)
SG	3 (43)	2 (29)	2 (29)	2 (29)
p	1.00	0.46	0.46	0.46
T2D, *n* (%)				
SADI-S	1 (14)	0 (0)	0 (0)	0 (0)
SG	1 (14)	0 (0)	0 (0)	0 (0)
p	1.00	–	–	–
MS, *n* (%)				
SADI-S	4 (57)	4 (57)	0 (0)	0 (0)
SG	6 (86)	4 (57)	1 (14)	0 (0)
p	0.56	1.00	1.00	–

Data are presented as mean ± SEM or number (%), as appropriate. SADI-S, single anastomosis duodeno-ileal bypass with sleeve gastrectomy; SG, sleeve gastrectomy; T2D, type 2 diabetes mellitus; MS, metabolic syndrome; TWL, total weight loss; EBMIL, excess BMI loss; SEM, standard error of mean. *$p < 0.05$ versus pre-operatory data within each surgical group.

during the MMTT were performed using a two-way ANOVA with Šidák's post hoc test. Fisher's exact test was used to compare nominal variables. Statistical analysis was carried in the GraphPad Prism version 9.4.1 for Windows (GraphPad Software, La Jolla, CA, USA). A p value <0.05 was considered statistically significant.

Results

Weight Loss Outcomes

BMI significantly decreased 12 months after SADI-S (48.6 ± 0.9 kg/m^2 vs. 28.2 ± 0.9 kg/m^2; $p < 0.01$) and SG (47.1 ± 1.0 kg/m^2 vs. 28.6 ± 0.8 kg/m^2, $p < 0.01$) (Table 2). %EBMIL followed the same trajectory and was not significantly different between groups (86.1 ± 3.9% vs. 84.6 ± 3.1%, SADI-S vs. SG), as well as TWL that reached its maximum at 12 months after surgery (41.6 ± 2.1% vs. 40.4 ± 1.7%, SADI-S vs. SG) (Table 2). There was no lost to follow-up.

Post-Operative Complications

Mortality rate was 0% for both procedures; 90-day morbidity was 14.3% (thrombophlebitis [n = 1, Clavien Dindo I]) and 14.3% (wound infection [n = 1, Clavien Dindo II]), for SADI-S and SG groups, respectively. Morbidity rate after the first 90 days was 0% in both groups. None of the patients reported symptoms compatible with dumping syndrome.

Micronutrients and Nutritional Deficiencies

There were no significant differences in micronutrient levels between patient groups, or in the rate of micronutrient deficiencies 12 months after surgery (data not shown).

Obesity Comorbidities

There was no significant difference in the rate of obesity comorbidities between the two groups at any time-point (Table 2). However, some patients had persistent hyperlipidemia (n = 4; n = 2 in each group), hypertension (n = 2 in SG group), and prediabetes (n = 1 in SG group), at 12 months of follow-up.

Fasting Glucose and Insulin Homeostasis

Fasting glucose, insulin, and C-peptide levels did not differ significantly between groups. Fasting ISR and insulin clearance were not significantly different between the two groups (Table 3).

Fasting Ghrelin Levels

Fasting ghrelin levels decreased significantly after both SG and SADI-S. No differences were observed between the surgical groups in none of the time-points (Table 3).

Response to the MMTT

There were no significant differences between the two groups in glucose, insulin, and C-peptide, nor in ISR and insulin clearance, at any time-point of the MMTT (Table 3). Post-operatively glucose, insulin, and C-peptide tended to peak at 30–45 min after meal in the SG group, while in the SADI-S group the same tended to peak at a later time-point, 45–60 min after the meal. It should be noticed that ISR was back to fasting at 120 min after meal, with no significant differences between groups (Table 3).

Table 3. Glucose ghrelin and insulin homeostasis before and after surgery

	Pre-op	3 months	6 months	12 months
Glucose				
Fasting, mg/dL				
SADI-S	101±4	86±3*	87±1	88±4*
SG	116±16	84±3*	83±4	90±3
p value	0.97	0.99	0.96	1.00
tAUC, mg/dL × min				
SADI-S	15,385±856	13,577±895	11,518±227*	11,186±477*
SG	16,181±2,479	13,824±551	11,169±611	12,414±819
p value	1.00	1.00	0.98	0.64
Insulin				
Fasting, pmol/L				
SADI-S	182.7±26.8	75.3±10.9	52.6±5.4*	40.0±7.2*
SG	129.0±16.3	47.6±5.8*	49.5±8.8*	38.9±4.5*
p value	0.59	0.31	1.00	1.00
tAUC, pmol/L × min				
SADI-S	88,762±11,586	90,278±12,494	77,945±10,808	58,257±8,935*
SG	77,314±11,939	64,952±6,989	55,302±7,322	48,034±7,216
p value	0.94	0.37	0.38	0.86
C-peptide				
Fasting, pmol/L				
SADI-S	715.9±113.6	523.1±75.8	377.9±51.5	329.1±20.9*
SG	589.4±44.6	406.4±63.0	383.3±45.6	360.0±33.7*
p value	0.94	0.88	1.00	0.99
tAUC, pmol/L × min				
SADI-S	137,830±11,821	199,236±34,831	166,314±29,042	159,417±15,479
SG	144,583±15,063	187,974±10,366	149,428±23,598	155,358±9,541
p value	0.99	1.00	0.99	1.00
ISR				
Fasting, pmol.kg^{-1}min^{-1}				
SADI-S	1.7±0.3	1.4±0.2	1.1±0.1	1.0±0.1*
SG	1.4±0.1	1.1±0.1*	1.2±0.1	1.1±0.1*
p	0.82	0.72	1.00	0.94
tAUC, pmol.kg^{-1}min^{-1} × min				
SADI-S	369.0±22.1	621.0±121.1	545.0±100.2	560.0±54.1
SG	386.9±48.7	587.1±38.0	489.5±84.6	535.5±31.8
p value	1.00	1.00	0.99	0.99
Insulin clearance				
Fasting				
SADI-S	0.010±0.001	0.020±0.003	0.022±0.003*	0.032±0.005*
SG	0.011±0.001	0.024±0.003	0.026±0.004	0.030±0.002*
p value	0.66	0.73	0.91	1.00
Post-prandial, 0–120 min				
SADI-S	0.005±0.001	0.007±0.001	0.008±0.002	0.011±0.002*
SG	0.005±0.001	0.009±0.001	0.010±0.002	0.012±0.002*
p value	0.90	0.10	0.87	0.99
HOMA-IR				
SADI-S	5.95±0.80	1.63±0.20*	1.76±0.38*	1.46±0.19*
SG	7.64±1.20	2.69±0.41	1.89±0.20*	1.46±0.30*
p value	0.30	0.11	1.00	0.81
HOMA-β, %				
SADI-S	221.00±53.54	157.90±25.00	158.30±11.95	88.46±9.62
SG	306.10±51.27	202.90±32.26	136.20±14.34*	105.50±17.19*
p value	0.30	0.58	0.47	0.11
Fasting ghrelin, pg/mL				
SADI-S	393.80±57.08	148.9±60.96*	257.8±102	220.2±49.21*
SG	371.80±70.73	146.2±45.52*	164.5±42.92*	182.8±29.00*
p value	0.84	0.97	0.81	0.72

Data are presented as mean ± SEM. SADI-S, single anastomosis duodeno-ileal bypass with sleeve gastrectomy; SG, sleeve gastrectomy; iAUC, incremental area under the curve; tAUC, total area under the curve; ISR, insulin secretion rate; HOMA-IR, homeostasis model assessment for insulin resistance (reference values: <1.85 for male and <2.07 for female [29]); HOMA-β, homeostasis model assessment for β-cell function (reference values: >67.1% for male and >86.2% for female [30]); SEM, standard error of mean. *$p < 0.05$ versus pre-operatory data within each surgical group.

Discussion

BPD/DS, a modified version by Hess and Marceau of the original BPD first described by Scopinaro [19, 20], is recognized to be the most effective bariatric surgery procedure for weight loss and comorbidity resolution, in patients with severe and super-obesity [21]. BPD/DS introduced the concept of a two-step approach for high-risk patients. Recognizing the technical simplicity and relative safety of using SG as the first step, along with its favorable outcomes, led to its establishment as a stand-alone technique in surgical practice [22]. Noticeably, SG is currently one of the procedures most frequently performed worldwide, despite the suboptimal results in patients with severe obesity [23]. In contrast, bariatric techniques with demonstrated effectiveness in this patient population, such as SADI-S and BPD/DS, continue to be marginally used by most surgeons around the world [24–26].

Despite the fact that patients with severe and super-obesity demand more aggressive bariatric surgery techniques to achieve effective weight loss, this also constitutes a high surgical risk population [4]. SADI-S represents a simplified version of BPD/DS and can also be used as a stepwise approach with SG as the first step [27]. However, it was unknown how weight loss achieved by SG as a first-step procedure compares to a single-step intervention, and what mechanisms and molecular adaptations underlie weight loss induced by the SG as the first-step procedure.

Our goal was to compare the weight trajectories of one- and two-step SADI-S during the first 12 months after intervention and take this opportunity to disentangle the contributions of each component of the procedure for the metabolic adaptation. By answering to these questions, we could gather a rationale for opting between one- or two-step intervention strategies whenever planning a SADI-S for the treatment of a patient with severe obesity.

In this study, we assessed pre- and post-operative post-prandial pancreatic hormonal response to a standardized meal of patients with severe obesity submitted to SADI-S and SG. This represented a leap forward, as previous assessments of molecular responses to a MMTT after SADI-S had neither gathered data for as many post-operative time-points nor compared to SG [6, 7, 28].

After surgery, both patient groups showed significant weight loss, which was not significantly different between groups. Even though mean BMI was above the normal threshold at the end of 12 months, homeostasis model assessment for insulin resistance and homeostasis model assessment for β-cell function were used as surrogate of insulin resistance and pancreatic β-cell function and experienced significant improvements in both groups. The fact that the study participants were predominantly euglycemic, apart from 1 single patient with prior diagnosis of diabetes at each study group who experienced clinical remission soon after surgery, and there were no patients with major metabolic imbalances along the study, not only reduced the risk of bias induced by the metabolic variability, but also enabled the analysis on how the metabolic and hormone profile to a meal challenge compared in individuals with normal glucose homeostasis, after each procedure.

Overall, our data suggest that in patients with severe obesity SADI-S and SG elicit similar adaptations of glucose and insulin homeostasis. This study reassured surgeons that selecting a two-step approach over the technically more demanding one-step SADI-S does not compromise metabolic results, as patients showed identical weight loss trajectories and MMTT responses 12 months following sleeve and SADI-S.

Ghrelin is an orexigenic hormone that is predominantly secreted at the gastric fundus, which is surgically removed in both SG and SADI-S. Therefore, it was anticipated that these procedures were likely to have a similar impact on the ghrelin levels and consequently appetite reduction.

Our data support the hypothesis that during the first year after surgery the restrictive component in addition to its molecular adaptations is the predominant mechanism underlying appetite reduction, weight loss, and improved glucose homeostasis profiles, observed after both procedures. Yet, it is reasonable to hypothesize that the intestinal component of a SADI-S is likely to have a more predominant effect later on after surgery.

Therefore, SG and SADI-S are positioned in the spectrum of one single surgical procedure: whether performed as a one- or two-step intervention, obesity treatment goals are met with non-inferiority when compared with one another. Micronutrient deficiencies, traditionally attributed to the hypoabsorption component, were not more likely to occur after SADI-S than SG.

The herein data are a valuable piece of information for the surgeon when facing a patient with class 3 obesity. Since performing a single-step SADI-S is technically more challenging, a two-step procedure is the preferred approach by most surgeons. However, there was the concern that the latter could jeopardize the weight loss outcomes in the first 12 months before completing the surgical technique and could not be as good as with a one-step strategy, as there were no data from RCTs that compared the efficacy of one- to two-step procedures.

This is the first study to provide evidence on weight loss outcomes of a head-to-head comparison of a single-step or two-step SADI-S. By conducting this study, we were able to get an answer to those questions and reassure patients and surgeons that weight loss in the first year after surgery is not hampered by this approach. Instead of having to decide between performing a one-step SADI-S or DS, we recommend a SG procedure to start with, which is expected to achieve similar weight loss and metabolic outcomes during the first post-operative year, setting aside surgical complexity. Nevertheless, whether similar weight loss outcomes will persist over the long-term is unknown.

Even though these findings have great significance, the present study harbors some limitations, namely, the small number of patients per group and the limited follow-up up time, since the post-operative evaluations did not proceed until weight loss stabilization. Additionally, a more comprehensive characterization of hormone profiles, including those known to impact on weight loss and glucose homeostasis, namely, glucagon, glucagon-like peptide-1, glucose-dependent insulinotropic polypeptide, was not conducted. Furthermore, understanding how the outcomes of patients undergoing either one- or two-step SADI-S are compared at 24 months after the first intervention is a highly sought-after question that remains to be answered.

Conclusion

Given that clinical outcomes and molecular profiles of patients submitted to SADI-S or SG are identical 1 year after surgery, these data reinforce the hypothesis that the restrictive component is the main driver underlying the post-operative changes. Furthermore, these data provide support for the bariatric surgeon decision, when faced with a patient with severe obesity, to safely opt for an SG as the first step of a SADI-S intervention, without jeopardizing the surgical outcomes.

Statement of Ethics

This study protocol was reviewed and approved by the Institutional Ethical Review Board (CA-110/2020-0t_MP/AC) from Unidade Local de Saúde de Entre o Douro e Vouga. The procedures conducted in this study involving human participants were in accordance with the ethical standards established by the institutional research committee. Moreover, they followed the principles outlined in the 1964 Helsinki Declaration and its subsequent amendments, or equivalent ethical standards. All participants signed written informed consent forms prior to any study intervention.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

Ana Marta Pereira and Diogo Moura contributed equally to the manuscript and contributed to the conception, investigation, methodology, data collection, and drafting the manuscript; Sofia S Pereira and Sara Andrade were involved in the investigation, formal analysis, and reviewing and editing the manuscript; Rui Ferreira de Almeida and Mário Nora were involved in the methodology, resources, and reviewing and editing the manuscript; Mariana P. Monteiro and Marta Guimarães were involved in the conceptualization, project administration, and reviewing and editing the manuscript.

Data Availability Statement

Anonymized data are available under request to the corresponding author with proper justification. Data are not publicly available due to ethical reasons. Further inquiries can be directed to the corresponding author.

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