OBES SURG (2017) 27:881–888
DOI 10.1007/s11695-016-2382-y

ORIGINAL CONTRIBUTIONS

A Comparative Study Examining the Impact of a Protein-Enriched Vs Normal Protein Postoperative Diet on Body Composition and Resting Metabolic Rate in Obese Patients after Sleeve Gastrectomy

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Published online: 27 September 2016

Abstract
Background We recently showed that an 8-week preoperative protein-enriched diet (PED) is associated with significant reductions in body weight and fat mass (FM) without significant loss of fat-free mass (FFM) in morbidly obese patients scheduled for laparoscopic sleeve gastrectomy (LSG).
Objectives The objective of this study is to evaluate the impact of PED vs a normal protein diet (NPD) on total weight loss (TWL), FM, FFM, and resting metabolic rate (RMR) in patients after LSG.
Methods Before LSG and at 3, 6, and 12 months after, we prospectively measured and compared total body weight (TBW), FM, FFM, and RMR in 60 male patients who received either a NPD (n = 30) with protein intake 1.0 g/kg of ideal body weight, or a PED (n = 30) with protein intake 2.0 g/kg of ideal body weight. Compliance in following the prescribed diet was determined with food frequency questionnaires in all patients. The impact of NPD and PED on renal function was also evaluated.
Results Despite non-significant variation in total body weight (TBW), FM decreased more significantly ($p < 0.01$) with the PED compared to the NPD. In addition, the PED group showed a significantly ($p < 0.01$) lower decrease in FFM and RMR when compared with the NPD group. Both groups showed high compliance in following the prescribed diets, without negative impact on renal function.
Conclusion PED is more effective than NPD in determining FM loss and is associated with a lower decrease in FFM and RMR, without interfering with renal function in male patients after LSG.

Keywords Obesity · Sleeve gastrectomy · Body composition · Fat-free mass · Protein intake

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Introduction

An important goal during weight loss is to maximize fat mass (FM) loss while preserving metabolically active fat-free mass (FFM) [1]. Maintaining adequate FFM is an important consideration when making dietary intake recommendations for weight loss because muscles play a central role in whole-body protein metabolism [2]. Additionally, a significant decrease in FFM may negatively affect the resting metabolic rate (RMR) [3], slow the rate of weight loss, and predispose to weight regain [4]. In 2010, Mettler et al. [5] demonstrated that consuming dietary protein at 2.3 g kg^{-1} day^{-1} was superior to 1.0 g kg^{-1} day^{-1} (recommended dietary allowance; RDA) for the maintenance of FFM in young athletes. Recently, Pasiakos et al. [6] demonstrated with volunteer military personnel from the U.S. army that consuming 1.6 g kg^{-1} day^{-1} (twice the RDA) is enough to protect FFM during short-term weight loss.

Laparoscopic sleeve gastrectomy (LSG) is one of the most performed bariatric surgery procedures for long-term treatment of morbid obesity [7–9] mainly because of its simple surgical technique that does not include any digestive anastomosis and leaves the continuity of the digestive tract intact. Furthermore, LSG results in weight loss and obesity-related comorbidities that are comparable to what is achievable with more complex procedure such as the Roux-en-Y gastric bypass [10–12]. Most nutritional guidelines suggest that after LSG, protein intake should be around 1 g/kg of ideal body weight per day [13, 14]. However, some studies reveal that using this amount of protein markedly changes FFM after LSG [15–17].

We have recently shown that in obese patients scheduled for LSG, an 8-week preoperative protein-enriched diet (PED) is associated with significant reductions in body weight and fat mass (FM) without a significant loss of fat-free mass (FFM) [18]. Therefore, the present study aims to evaluate the clinical impact of PED vs normal protein diet (NPD) on total weight loss (TWL), FM, FFM, and RMR in morbidly obese patients that underwent LSG.

Materials and Methods

Patient Selection

We prospectively assessed 60 obese men that consecutively underwent LSG at our university hospital between 2011 and 2014. All patients fulfilled the criteria established by the International Federation for Surgery of Obesity for surgical treatment [19]. Only men were included to reduce hormonal interference (i.e., changes in hydration status related to menstrual cycle or menopausal conditions). The inclusion criteria were: body mass index (BMI) ≥40 kg/m^2, or ≥35 kg/m^2 with comorbidities related to obesity and age between 18 to 65 years. Patients with previous gastrointestinal surgery, gastro-esophageal reflux disease, digestive and/or inflammatory bowel diseases, mental illness, and inability to comply with the PED or NPD for religious reasons or the presence of chewing or swallowing disorders, were excluded from the study groups. Patients after LSG were randomized into two groups: the NPD group ($n = 30$) that followed a NPD (protein intake 1.0 g/kg ideal body weight diet) [13, 14] and the PED group ($n = 30$) that followed a PED (protein intake 2.0 g/kg ideal body weight diet) [18].

Preoperative Characteristics of the Study Population

Preoperative age, height, and BMI were 41 ± 6.2 years, 1.76 ± 2.4 cm, and 40.7 ± 5.3 kg/m^2 in the NPD group, and 43 ± 5.5 years, 1.78 ± 3.4 cm, and 42.1 ± 6.2 kg/m^2 for the PED group, respectively. Preoperative total body weight (TBW), FM (% and kg), FFM (% and kg), and RMR are reported in Fig. 1.

NPD and PED Characteristics

Consistent with current guidelines [14, 15], after discharge, patients assumed a liquid diet, a puree-based diet after 15 days, and a soft solid food diet after 4 weeks. To ensure patients of both groups consumed a similar diet, we developed three NPD and PED postoperative meal plans (each consisting of 1200 kcal/day) using foods and ingredients reported in Fig. 2: plan 1 (months 1 to 4), plan 2 (months 5 to 8), and plan 3 (months 9 to 12) using Nutrigeo 8 software (Progeo, Ascoli Piceno, Italy). NPD and PED composition provided a protein intake of 1.0- and 2.0-g/kg ideal body weight, respectively (Fig. 2). In both NPD and PED groups, ideal body weight was calculated using a BMI of 22.5 kg/m^2 and, based on preoperative characteristics, was fixed on 70 kg for the NPD group and 71.5 kg for the PED group. In both groups, the diet's fat percentage was fixed at 15 %; the NPD was consequently more rich in carbohydrates (61.7 %) than the PED (37.3 %). Macronutrient composition of both the NPD and PED are reported in Fig. 2. In addition, after discharge, all patients received the same commercially available mineral and vitamin supplement (WLS Optimum, Fit for me, Orte, Italy) specially formulated for obese patients and/or those who have undergone surgery for obesity [20].

Anthropometric, Body Composition and RMR Measurements

Measurements were performed before LSG, and at three, six, and 12 months after. Body weight (in kilogram) and height (in centimeter) were determined under standard conditions. Height was measured using a Seca 206 mechanical measuring tape (Intermed, Milano, Italy); body weights were assesed by Seca 869 flat digital scale (capacity 250 kg, Intermed).

Patients' body composition were measured by bioelectrical impedance assay (BIA) using the Jawon IOI 353 Segmental Body Composition Monitor (Cosmed, Rome, Italy). The

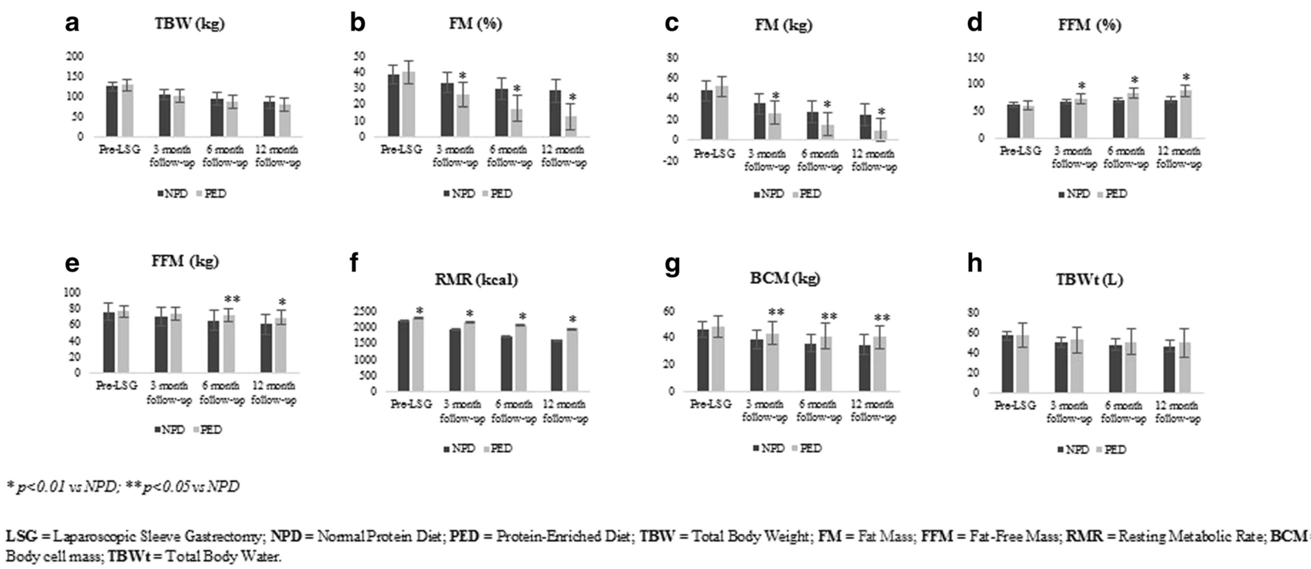

Fig. 1 Post-LSG-PED vs post-LSG-NPD: a comparison between patient's anthropometric data, body composition, resting metabolic rate, body mass cell (BCM), and total body water (TBWt), preoperatively and at postoperative months 3, 6, and 12

instrument used is the last generation in body composition analysis, use the latest multi-frequency technology and it is in compliance with the requirements of the Directive 90/384 /EEC for weighing with non-automatic devices in the medical sector and the Directive 93/42/EEC for medical devices.

To perform an appropriate analysis, as we previously reported [18], all patients were required to comply with these conditions prior to the BIA: no food ingestion for at least 4 h, minimal intake of 2 L of water the day before, no physical activity for at least 8 h, no coffee or alcoholic beverage consumption during at least 12 h, and no diuretic use for at least 24 h. Patients were also asked to empty their bladder immediately prior to the BIA test. Patient's RMR were measured by indirect calorimetry using Fitmat PRO monitor (Cosmed). Examinations were performed from 8:00 to 10:00 am in the same room under thermos neutral conditions, in order to reduce diurnal variation between subjects [21–24]. Measurements were performed at a duration of 15 min following a prior 5- to 10-min test.

Dietary Compliance Assessment Methods

Nutritional assessment and dietary counseling were scheduled at 3, 6, and 12 months after LSG. Dietary assessments were primarily performed using questionnaires (3-day estimated food records and 72-h recalls) [25]. Nutrient intakes were

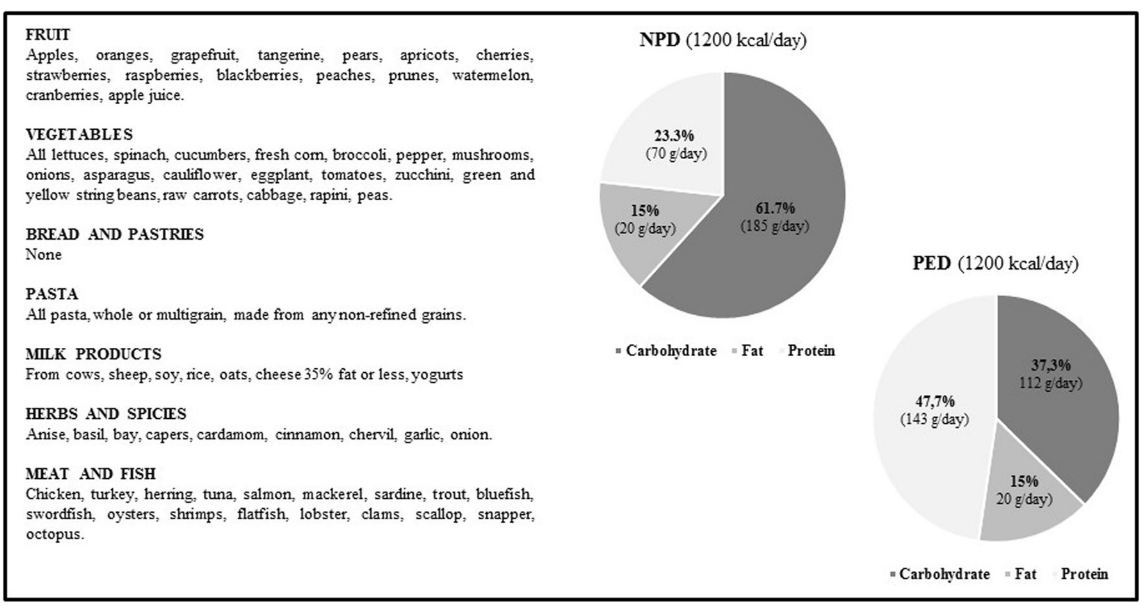

Fig. 2 Normal protein diet (NPD) and protein-enriched diet (PED) food plans: food and ingredients used, energy, and macronutrient distribution

calculated from the 72-h recalls and 3-day dietary records (Sunday to Tuesday; breakfast to bedtime) using Nutrigeo 8 software.

Measurement of Blood Parameters

Biochemical and hematologic tests were conducted preoperatively and at 12 months after LSG in both groups (Table 1).

Statistical Analysis

The effects of post-LSG NPD and PED program were directly compared by using the paired sample *t* test for continuous variables (Graph Pad Software, La Jolla, CA, USA). The pattern of TBW, FM, FFM, and RMR changes during the period study was expressed as a percentage and plotted over time. Simple bivariate analysis was used to assess the correlation between FFM loss and protein intake. A p value <0.05 was considered statistically significant.

Results

Impact of NPD and PED on TBW, FM, FFM, and RMR

Before surgery, NPD and PED groups had a normal protein preoperative diet and were comparable in terms of TBW, FM, FFM, whereas PED group RMR was significantly ($p < 0.01$) higher than the NPD group (Fig. 1 a–f). Three patients dropped out of the NPD group and two from the PED group. As expected, we observed that TBW and FM (in percent and in kilogram) markedly changed after surgery in both groups (Fig. 1a–c). However, we did not observe any significant difference between the two groups in terms of TBW lost. Patients

Table 1 Patient's clinical parameters at baseline and after LSG (12 months)

Clinical parameters	Diet	Pre-LSG	Post-LSG (follow-up 12 months)	Difference (%)	*t* Student value	*p* value
Triglycerides (mg/dL)	NPD	234 ± 38.6	124 ± 25.9	−47	18.3	<0.01*
	PED	215 ± 22.1	103 ± 15.3	−52.1	32.3	<0.01*
Total Cholesterol (mg/dL)	NPD	241 ± 16.4	174 ± 33.4	−30.7	13.9	<0.01*
	PED	223 ± 29.8	145 ± 21.5	−35	16.4	<0.01*
HDL (mg/dL)	NPD	41 ± 6.5	61 ± 9.3	+48	13.6	<0.01*
	PED	36 ± 7.9	73 ± 16.5	+102	15.7	<0.01*
LDL (mg/dL)	NPD	153 ± 27.9	88.2 ± 17.4	−42.4	15.3	<0.01*
	PED	144 ± 27.9	51.4 ± 27.9	−64.6	18.2	<0.01*
Glucose (mg/dL)	NPD	128 ± 11.7	82 ± 11.9	−36	21.3	<0.01*
	PED	136 ± 21.4	72 ± 16.9	−47.1	18.2	<0.01*
Insulin (mU/L)	NPD	11.9 ± 5.9	4.3 ± 8.9	−63.9	5.5	<0.01*
	PED	9.3 ± 5.9	3.3 ± 11.3	−64.6	3.6	<0.01*
GOT (U/L)	NPD	52 ± 6.1	29 ± 8.4	−44.2	17.2	<0.01*
	PED	42 ± 3.9	21 ± 9.4	−50	15.9	<0.01*
GPT (U/L)	NPD	41 ± 9.3	31 ± 11.6	−24.4	5.2	<0.01*
	PED	39 ± 9.3	19 ± 9.9	−51.3	11.4	<0.01*
GGT (U/L)	NPD	29 ± 18.2	19 ± 23.4	−34.5	2.6	<0.01*
	PED	31 ± 9.2	20 ± 16.7	−35.5	4.5	<0.01*
Creatinine (mg/dL)	NPD	1.2 ± 8.5	0.98 ± 7.2	−18.4	0.15	0.8787**
	PED	0.98 ± 6.9	0.91 ± 11.4	−7.2	0.04	0.9676**

Data are reported as mean ± standard deviation (SD), and difference expressed in percentage (%)

LSG laparoscopic sleeve gastrectomy, NPD normal protein diet, *PED* protein-enriched diet, *HDL* high-density lipoprotein, *LDL* low-density lipoprotein, *GOT* glutamic oxaloacetic transaminase, *GPT* glutamic pyruvic transaminase, *GGT* gamma-glutamyl transferase

Statistical analysis was performed using paired sample test by *t* test (Graph Pad Software, Inc.)

Any p value lesser than 0.05 was statistically significant

Any p value lesser than 0.01 was extremely statistically significant

*$p < 0.01$;

**$p \geq 0.01$, $p \geq 0.05$—by conventional statistical criteria, this difference is not statistically significant who followed the PED lost significantly more FM (in percent and in kilogram) at 3 ($p < 0.01$), 6 ($p < 0.01$), and 12 months after surgery ($p < 0.01$) than patient who followed the NPD. On the contrary, patients that followed the PED lost less FFM (in percent) at 3 ($p < 0.01$), 6 ($p < 0.01$), and 12 months after surgery ($p < 0.01$), than patients who followed the NPD (Fig. 1d). PED patients lost significantly less FFM (in kilogram) at 6 ($p < 0.05$) and 12 months ($p < 0.01$) after surgery (Fig. 1e).

The FFM loss was highly correlated with the protein intake ($r = 0.61$; $p < 0.001$). Finally, patients that followed the PED showed a significantly higher RMR at 3 ($p < 0.01$), 6 ($p < 0.01$), and 12 months ($p < 0.01$) after LSG than patients who followed the NPD (Fig. 1f).

Three-Day Estimated Food Records Vs 72-H Recalls

No significant differences in the estimated nutrient intake were observed between the 72-h recalls and the 3-day estimated food records in both NPD and PED groups. Values for energy intake (expressed in kilocalorie per day) and all macronutrients reported during the 72-h recalls were strictly similar to those of the 3-day estimated records, indicating a high patient's compliance of following the prescribed diets in both group studies (Tables 2 and 3).

Impact of NPD and PED on patient's Clinical Parameters

Both NPD and PED patients showed a marked improvement in several clinical parameters, including liver enzyme levels, glycemic, and lipid profiles, while no change on renal function was observed (Table 1).

Discussion

This study indicates that PED is more effective than NPD in determining FM loss and is associated with a lower decrease in FFM and RMR, without interfering with renal function in male morbidly obese patients undergoing LSG. In accordance with Belfiore et al. [17], we found that NPD is associated with a remarkable loss of FFM at 3 and 6 months after surgery. Indeed, Belfiore et al. [17] reported the proportion of body mass loss attributable to FFM to be 12 and 15.7 % at 3 and

Table 2 Mean and standard deviation (SD) of daily intake of macronutrient from the 3-day estimated food record and the 72-h recall of the 30 participants consuming a normal protein diet (NPD)

Parameter	NPD	3-day estimated food record	72-h recall	p
	Food plan 1	Follow-up 3 months	Follow-up 3 months	
Energy (kcal)	1200	1146.5 ± 4.80	1171 ± 3.91	NS
Protein (g)	70 (280 kcal)	66 (264 kcal) ± 2.2	68 (272 kcal) ± 2.8	NS
Protein (%)	23.3	23 ± 6.1	23.2 ± 11.4	NS
Carbohydrate (g)	185 (740 kcal)	179 (716 kcal) ± 9.3	173 (692 kcal) ± 7.1	NS
Carbohydrate (%)	61.7	62.4 ± 10.6	59 ± 14.4	NS
Fat (g)	20 (180 kcal)	18.5 (166.5 kcal) ± 7.7	23 (207 kcal) ± 6.9	NS
Fat (%)	15	14.6 ± 9.2	18.8 ± 9.8	NS
	Food plan 2	Follow-up 6 months	Follow-up 6 months	
Energy (kcal)	1200	1181 ± 1.60	1162 ± 1.81	NS
Protein (g)	70 (280 kcal)	74 (296 kcal) ± 7.2	71 (284 kcal) ± 8.6	NS
Protein (%)	23.3	25 ± 8.1	24.4 ± 9.4	NS
Carbohydrate (g)	185 (740 kcal)	181 (724 kcal) ± 8.3	182.5 (730 kcal) ± 5.3	NS
Carbohydrate (%)	61.7	61.3 ± 10.6	62.8 ± 10.7	NS
Fat (g)	20 (180 kcal)	17.8 (160.2) ± 5.7	16.4 (147.6 kcal) ± 8.9	NS
Fat (%)	15	13.7 ± 11.7	12.8 ± 16.8	NS
	Food plan 3	Follow-up 12 months	Follow-up 12 months	
Energy (kcal)	1200	1210 ± 3.90	1225 ± 6.21	NS
Protein (g)	70 (280 kcal)	71 (284 kcal) ± 5.2	72.5 (290 kcal) ± 8.6	NS
Protein (%)	23.3	23.5 ± 8.1	23.7 ± 11.4	NS
Carbohydrate (g)	185 (740 kcal)	180 (720 kcal) ± 3.3	179 (716 kcal) ± 4.1	NS
Carbohydrate (%)	61.7	59.5 ± 10.6	58.4 ± 14.4	NS
Fat (g)	20 (180 kcal)	22.9 (206 kcal) ± 11.7	24.3 (218.7) ± 6.9	NS
Fat (%)	15	17 ± 11.7	17.9 ± 12.2	NS

NS by conventional statistical criteria, this difference is not statistically significant ($p \geq 0.01$, $p \geq 0.05$)

Table 3 Mean and standard deviation (SD) of daily intake of macronutrient from the 3-day estimated food record and the 72-h recall of the 30 participants consuming a protein-enriched diet (PED)

Parameter	PED	3-day estimated food record	72-h recall	*p*
	Food plan 1	Follow-up 3 months	Follow-up 3 months	
Energy (kcal)	1200	1191 ± 6.2	1194 ± 8.7	NS
Protein (g)	143 (572 kcal)	140 (560 kcal) ± 4.4	145 (580 kcal) ± 7.7	NS
Protein (%)	47.7	47 ± 8.3	48.6 ± 14.4	NS
Carbohydrate (g)	112 (448 kcal)	108 (432 kcal) ± 7.6	105 (420 kcal) ± 9.7	NS
Carbohydrate (%)	37.3	36.3 ± 9.8	35.2 ± 10.6	NS
Fat (g)	20 (180 kcal)	22.1 (199 kcal) ± 4.5	21.5 (194 kcal) ± 5.9	NS
Fat (%)	15	16.7 ± 4.1	16.2 ± 5.8	NS
	Food plan 2	Follow-up 6 months	Follow-up 6 months	
Energy (kcal)	1200	1186 ± 3.6	1194 ± 3.4	NS
Protein (g)	143 (572 kcal)	145 (580 kcal) ± 4.4	144 (576 kcal) ± 5.6	NS
Protein (%)	47.7	48.9 ± 11.1	48.2 ± 12.1	NS
Carbohydrate (g)	112 (448 kcal)	115 (460 kcal) ± 9.6	113 (452 kcal) ± 3.5	NS
Carbohydrate (%)	37.3	38.8 ± 13.8	37.9 ± 7.1	NS
Fat (g)	20 (180 kcal)	16.2 (145.8) ± 8.7	18.4 (166 kcal) ± 9.9	NS
Fat (%)	15	12.3 ± 16.5	13.9 ± 18.1	NS
	Food plan 3	Follow-up 12 months	Follow-up 12 months	
Energy (kcal)	1200	1225 ± 9.3	1235 ± 2.6	NS
Protein (g)	143 (572 kcal)	150 (600 kcal) ± 7.2	152 (608 kcal) ± 6.6	NS
Protein (%)	47.7	48.9 ± 11.8	49.2 ± 15.5	NS
Carbohydrate (g)	112 (448 kcal)	106 (424 kcal) ± 7.3	108 (432 kcal) ± 10.1	NS
Carbohydrate (%)	37.3	34.6 ± 4.6	35 ± 6.6	NS
Fat (g)	20 (180 kcal)	22.3 (201 kcal) ± 5.75	21.7 (195 kcal) ± 10.9	NS
Fat (%)	15	16.5 ± 17.1	15.8 ± 18.3	NS

NS by conventional statistical criteria, this difference is not statistically significant ($p \geq 0.01$, $p \geq 0.05$)

6 months after LSG, respectively. In the present study, the proportion of body mass loss attributable to FFM, at 3, 6, and 12 months after LSG were 8.5, 13.8, and 19.8 %, respectively. Although Belfiore at al. [17] suggested that FFM loss took place because of poor patient compliance in following the postoperative diet, we found that FFM loss was independent of patient compliance. In fact, despite the NPD group being strictly compliant, FFM loss was significantly higher than in the PED group, suggesting that the significant reduction in FFM observed is mainly attributable to protein intake deficiency (Tables 2 and 3). Furthermore, our findings are in accordance with those recently reported by Schollenberger et al. [26] that showed that postoperative protein supplement might facilitate body fat loss, and protect against FFM wasting, without negative impact on renal function. In addition, and more importantly, to the best of our knowledge, this is the first study that demonstrates how the preservation of FFM during the LSG postoperative weight loss period has a positive impact on RMR. The present study suggests that a post-LSG PED induces a larger reduction of FM without a significant drop of FFM and RMR when compared to a NPD, highlighting the importance of a high protein diet to maintain FFM.

Considering that bariatric surgery deeply effects FFM, as suggested by Thibault et al. [27], one of the key nutritional issues to measure body before and after surgery to quantify these changes, and that protein and general diet composition should be adjusted to manage the risk of FFM depletion after surgery. In the present study, body composition was measured by BIA. We were aware that BIA in severely obese patients has been criticized because these patients may have altered electrical properties in their body tissues, which could cause an overestimation of FFM and an underestimation of FM [28, 29]. However, several studies conducted in obese patients validate the use of BIA for the measure of body composition, indicating that it is reliable and reproducible [18, 30–32]. However, we also monitored the changes in total body water (TBWt) and body cellular mass (BCM) in both groups by BIA (Fig. 2). In accordance with previous reports [17], we observed 3 months after surgery, a significant ($p < 0.05$) decrease in BCM (Fig. 2g) in the NPD group with no significant changes in TBWt (Fig. 2h). On the contrary, we show for the first time that a postoperative enriched-protein diet is also able to positively impact BCM. In particular, in the PED group we observed a significant ($p < 0.05$) increase in BCM (Fig. 2g), with no changes in TBWt at 3, 6 and 12 months after surgery

(Fig. 2h). Considering that BCM is an important factor in determining RMR [33], and has also been reported as a valuable indicator of nutritional status [34], our data suggests that a postoperative protein-enriched diet is able to better preserve RMR by positively impacting the FFM and BCM.

Finally, PED appears safe, considering that not only is it associated to an important improvement in patient's clinical status (Table 1) but also seems not to affect renal function. The present study has certain limitations. First, despite after discharge the physical activity was encouraged, we do not directly measure it. Secondarily, FM and FFM were only measured by BIA and were not supplemented with additional comparative measures. We are aware that measurements of body composition by others techniques, such as computed tomography (CT), can be more accurate. However, it would have been impossible to do a CT scan in most of the studied patients because of their weight. Furthermore, CT scan is not cost-effective and radiation exposure would not be acceptable for ethical issues. Furthermore, BIA has several advantages over other methods, such as high safety, noninvasiveness, low cost, ease of use, high reproducibility, and adaptability to medical routine. Second, this was a comparative cohort study involving a gender-biased sample. The reason is because, as reported in a recent review by Mialich and coworkers [35], standardization of measurement conditions is essential for obtaining accurate and reproducible BIA. Various individual factors have been shown to influence BIA. Overall, the within-subject total body impedance variability is higher in women, which appears to be due to changes in hydration status related to menstrual cycle [35].

Conclusion

Based on the present findings, we are able to support the hypothesis that in patients undergoing LSG, PED is more effective than NPD in determining FM loss and is associated with a significantly lower decrease in FFM and RMR, without interfering with renal function. All subjects showed a high compliance in following the prescribed diet and no unfavorable anthropometric, biochemical, or clinical outcomes were found.

These results should be confirmed in a larger randomized trial.

Compliance with Ethical Standards

Conflict of Interest The authors declare no conflicts of interest.

Informed Consent Written informed consent was obtained for each individual participant included in the study.

Ethical Approval All procedures performed in this study were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Declaration of Helsinki and its later amendments. *Research Registry Identifier Number* 1566.

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