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ORIGINAL CONTRIBUTIONS

Nutritional Status Prior to Laparoscopic Sleeve Gastrectomy Surgery

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Abstract

Background Two main causes for nutrient deficiencies following bariatric surgery (BS) are pre-operative deficiencies and favoring foods with high-energy density and poor micronutrient content. The aims of this study were to evaluate nutritional status and gender differences and the prevalence of nutritional deficiencies among candidates for laparoscopic sleeve gastrectomy (LSG) surgery.

Methods A cross-sectional analysis of pre-surgery data collected as part of a randomized clinical trial on 100 morbidly obese patients with non-alcoholic fatty liver disease (NAFLD) admitted to LSG surgery at Assuta Medical Center between February 2014 and January 2015. Anthropometrics, food intake, and fasting blood tests were evaluated during the baseline visit.

Results One-hundred patients completed the pre-operative measurements (60 % female) with a mean age of 41.9 ± 9.8 years and a mean BMI of 42.3 ± 4.7 kg/m^2. Pre-operatively, deficiencies for iron, ferritin, folic acid, vitamin B1, vitamin B12, vitamin D, and hemoglobin were 6, 1, 1, 6, 0, 22, and 6 %, respectively. Pre-surgery, mean energy, protein, fat, and carbohydrate intake were 2710.7 ± 1275.7 kcal/day, 114.2 ± 48.5, 110.6 ± 54.5, and 321.6 ± 176.1 gr/day, respectively. The intakes for iron, calcium, folic acid, vitamin B12, and vitamin B1 were below the Dietary Reference Intake (DRI) recommendations for 46, 48, 58, 14, and 34 % of the study population, respectively.

Conclusion We found a low prevalence of nutritional deficiencies pre-operatively except for vitamin D. Most micronutrient intake did not reach the DRI recommendations, despite

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high-caloric and macronutrient intake indicating a poor dietary quality.

Keywords Obesity · Bariatric surgery · Micronutrient deficiencies · Dietary supplements · Food intake

Introduction

Bariatric surgery (BS) is currently the most effective treatment modality for obesity and its associated metabolic complications [1]. The main benefits of this intervention include prolonged weight loss, improvement of associated comorbidities, and quality of life [2]. The total number of BS performed worldwide in 2013 were 468,609; 37 % of them were laparoscopic sleeve gastrectomy (LSG) surgeries [3]. In Israel, 11, 452 people with morbid obesity underwent BS in 2013 and LSG was the leading procedure [3]. All candidates for BS undergo pre-operative nutritional evaluation, including micronutrient measurements [4]. It was previously shown that if micronutrient deficiencies are not detected and corrected, they may influence post-operative morbidity and even mortality [5]. According to recently published studies, the pre-operative deficiency prevalence for vitamin B12 is 13–18 %, for iron is 8–47 %, for folic acid is 0–32 %, for vitamin D is 25–99 %, and for vitamin B1 is 0–29 % [5–9].

Causes of nutritional deficiencies in obesity are multifactorial and include high intake of foods with high caloric density and low nutritional quality, defective storage and bioavailability of some nutrients (e.g., vitamin D), increased hepcidin synthesis leading to reduce iron absorption due to chronic inflammation, and small intestinal bacterial overgrowth which may consume vitamin B1 and B12 and fat-soluble vitamins leading to their absence [10].

Causes and mechanisms of nutrient deficiencies following BS are also multifactorial and are influenced by type of procedure, pre-operative deficiencies, sustained post-operative vomiting, food intolerance, modified eating behavior, and non-adherence to dietary and supplement recommendations [10].

The aims of our study were to evaluate and to compare between genders, dietary intake, and micronutrient deficiencies among 100 candidates for LSG surgery with morbid obesity.

Materials and Methods

A cross-sectional analysis of pre-surgery data was collected as part of a randomized clinical trial (RCT) of 6-month treatment with probiotic vs. placebo among 100 non-alcoholic fatty liver disease (NAFLD) patients who underwent LSG surgery at the Assuta Medical Center from February 2014 to January 2015. Inclusion criteria were age between 18 and 65 years old, BMI > 40 or BMI > 35 kg/m^2 with comorbidities, approval of the Assuta hospitals' bariatric multidisciplinary team to undergo BS (the team includes registered dietitian, social worker/phycologist, internist/ endocrinologist, and surgeon), ultrasound-diagnosed NAFLD, and ability to sign an informed consent. Major exclusion criteria were fatty liver suspected to be secondary to hepatotoxic drugs, excessive alcohol consumption [11], mental illness or cognitive deterioration, and previous BS. Diabetic patients who were treated with anti-diabetic medications other than treatment with metformin at a stable dose for at least 6 months exclusively were also excluded.

Prior to their meeting with the bariatric multidisciplinary team, all patients must be evaluated and cleared by a registered dietitian. The registered dietitian assesses each patient's individual nutritional needs and food intake history, reviews proper nutrition, and discusses protein intake, and vitamin and mineral supplementation needs post-surgery. All patients get a recommendation by the registered dietitian to use supplements if nutritional deficiencies are detected. In addition, 2–3 weeks before the surgery, all patients are recommended to follow a low-carbohydrate diet and during that time, to take a daily multivitamin supplement [12].

The baseline evaluations were performed on average 24 ± 12 days pre-surgery. Medical history for comorbidities was obtained from the patients' medical records.

Biochemical Evaluation

Each participant underwent biochemical testing, following a 12 h fast, for lipid panel, glucose, HbA1C, insulin, C-reactive protein (CRP), vitamin B1, vitamin B12, vitamin D, iron, ferritin, folic acid, and hemoglobin (Hb). Patients were asked not to take supplements a day before blood was drawn in order not to cause spuriously elevated levels. Deficiency of a vitamin or a mineral was defined as a plasma level below the reference range recommended by the kit manufacturer (Fig. 1).

Anthropometry Measurements

Weight and height were measured on a digital medical scale, and waist circumference (WC) was measured twice at the level of the umbilicus by a single surveyor. BMI was calculated using weight (in kilograms) divided by the height squared (in square meter).

Dietary Intake Evaluation

Patients filled out a detailed semi-quantitative food frequency questionnaire (FFQ) reporting their habitual nutritional intake in the past year. The FFQ was assembled by the Food and Nutrition Administration, Ministry of Health, and was

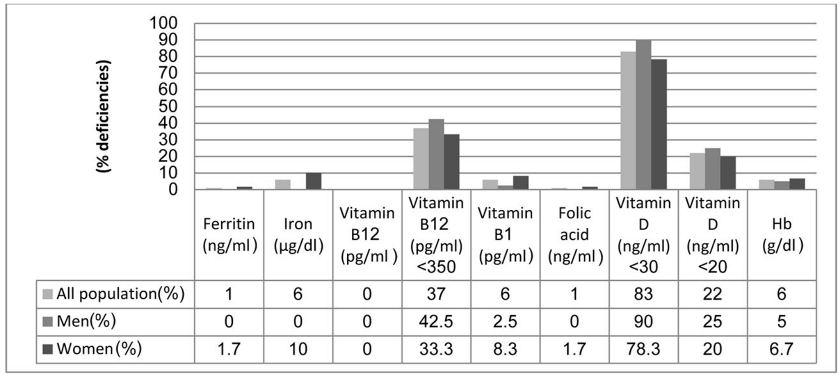


Fig. 1 Prevalence of micronutrient deficiencies

previously described in detail [13, 14]. It was adjusted for the current study needs. Caloric, macronutrient, and micronutrient intake were assessed based on the Israeli nutrient software "Zameret," which was developed by the Israeli Ministry of Health, and compared to recommended values of the Dietary Reference Intake (DRI) recommendations [15–18].

Statistical Methods

Statistical analyses were performed using SPSS version 22 (SPSS Inc., Chicago, IL, USA) software. The Kolmogorov-Smirnov test was used to assess whether the data were normally distributed. Results were expressed as mean ± standard deviation (SD) and/or by percentage. To test differences in continuous variables between two groups, the independent sample t test was performed. Associations between nominal variables were performed with the Pearson's chi-squared test. $P < 0.05$ was considered statistically significant for all analyses.

Results

One-hundred patients completed the pre-operative measurements (60 % female). Their mean age was 41.9 ± 9.8 years and the mean pre-operative BMI was 42.3 ± 4.7 kg/m^2; 13 % were diabetic (eight treated with metformin), 59 % had dyslipidemia, and 21 % had hypertension (Table 1). Fifteen patients (15 %) began a low-carbohydrate diet with the additional multivitamin supplementation 3–10 days before the baseline measurements.

Pre-Surgery Nutritional Deficiencies

Pre-operative nutritional deficiencies were found in 6, 1, 0, 37, 1, and 6 % of the participants for iron, ferritin, and vitamin B12 for the laboratory cutoff (<175 pg/ml) and vitamin B12, folic acid, and vitamin B1 for the cutoff <350 pg/ml, respectively. Vitamin D levels were categorized as deficiency (<20 ng/ml) found in 22 % and insufficiency (<30 ng/ml) found in 83 % of the participants (Tables 2 and 3). Hemoglobin was low in 6 % of the patients (Fig. 1). No significant differences were found between genders for all micronutrient

Table 1 Baseline characteristics of the study sample

Parameter[a]	All population	Men (n = 40)	Women (n = 60)
Age (year)	41.9 ± 9.8 (21.0–63.0)	43.5 ± 9.4 (24.0–63.0)	40.7 ± 10.0 (21.0–60.0)
Weight (kg)	121.2 ± 19.5 (86.0–203.5)	135.2 ± 19.6 (106.8–203.5)	111.9 ± 12.8 (86.0–149.6)
Height (m)	1.69 ± 0.09 (1.52–1.93)	1.78 ± 0.06 (1.66–1.93)	1.63 ± 0.05 (1.52–1.74)
BMI (kg/m^2)	42.3 ± 4.7 (34.6–60.0)	42.6 ± 5.1 (34.6–60.0)	42.1 ± 4.4 (34.7–55.0)
WC (cm)	124.3 ± 12.3 (101.0–164.0)	131.0 ± 11.9 (109.0–164.0)	119.9 ± 10.5 (101.0–150.0)
Type 2 diabetes (%)	13.0	22.5	6.7
Dyslipidemia (%)	59.0	65.0	55.0
Hypertension (%)	21.0	27.5	16.7
Thalassemia minor (%)	2.0	1.0	1.0
Current smoker (%)	9.0	7.5	10

BMI body mass index, *WC* waist circumference

[a] Values are expressed as the average ± standard deviation (range)

Table 2 The prevalence of pre-operative treatment with different dietary supplements

Parameter	All population (%)	Men (%) $n=40$	Women (%) $n=60$	P value
Multivitamin	12.0	10.0	13.3	0.615
Vitamin D	48.0	32.5	58.3	0.011
Vitamin B12	19.0	15.0	21.7	0.405
Folic acid	14.0	10.0	16.7	0.347
Iron	10.0	5.0	13.3	0.174
Other supplements	4.0	5.0	3.3	0.677
Total vitamins and minerals	59.0	47.5	66.7	0.056

deficiencies. Only 10 % of women and 18.3 % of men did not present any nutritional deficiency pre-surgery.

Pre-Surgery Nutritional Intake

Mean energy, protein, protein intake per kilogram of body weight, and fat and carbohydrate intake were 2710.7 ± 1275.7 kcal/day, 114.2 ± 48.5 gr/day (17 % of calories), 1.0 ± 0.4 gr/day, 110.7 ± 54.5 gr/day (36 % of calories), and 321.6 ± 176.1 gr/day (47 % of calories), respectively, which is above the DRI recommendations. Mean fiber intake was 28.1 ± 16.5 gr/day for men, which is below the DRI recommendations, and 34.8 ± 24.3 gr/day for women, which is adequate to the DRI recommendations. Mean sugar-sweetened beverages

Table 3 Biochemical parameters and comparison between men and women

Parameter[a]	All population	Men	Women	P value
Glucose (mg/dl) 70–100	91.3 ± 22.6	97.9 ± 30.8	86.9 ± 13.4	0.039
Insulin (mcu/ml) 5–25	26.0 ± 14.9	30.1 ± 16.6	23.2 ± 13.0	0.023
HbA1C (%) Diabetes ≥6.5 %	5.8 ± 0.7	6.2 ± 1.0	5.6 ± 0.4	0.001
Tg (mg/dl) 50–150	151.2 ± 79.9	162.5 ± 74.7	143.6 ± 82.8	0.250
TC (mg/dl) 150–200	187.8 ± 33.2	179.8 ± 33.0	193.1 ± 32.6	0.050
HDL (mg/dl) M 35–70; W 39–90	47.1 ± 15.6	38.4 ± 8.0	52.9 ± 16.7	<0.001
LDL (mg/dl) 60–160	111.4 ± 26.2	109.1 ± 26.4	112.9 ± 26.2	0.471
CRP (mg/l) 0–5	13.1 ± 11.3	9.3 ± 7.8	15.6 ± 12.6	0.003
Iron (μg/dl) 40–150	75.3 ± 27.4	83.9 ± 26.0	69.6 ± 26.9	0.010
Ferritin (ng/ml) M 14–163; W 7.1–151	110.0 ± 100.6	179.1 ± 118.3	63.9 ± 48.4	<0.001
Vitamin B12 (pg/ml) 175–961	419.1 ± 176.7	390.6 ± 108.0	438.1 ± 209.2	0.189
Vitamin B1 (pg/ml) 32–95	58.8 ± 15.3	63.1 ± 15.2	56.0 ± 14.8	0.023
Folic acid (ng/ml) 2.6–17.1	9.1 ± 4.4	9.0 ± 4.5	9.2 ± 4.4	0.819
Vitamin D (ng/ml) Insufficiency 20–30 Deficiency <20	24.5 ± 6.3	24.0 ± 6.1	24.8 ± 6.4	0.538
Hb (g/dl) M 13.2–17; W 11.7–15.5	13.9 ± 1.4	15.0 ± 1.0	13.1 ± 1.0	<0.001

TC total cholesterol, *LDL-C* low-density lipoprotein cholesterol, *HDL-C* high-density lipoprotein cholesterol, *Tg* triglycerides, *CRP* C-reactive protein, *HbA1C* hemoglobin A1c, *Hb* hemoglobin

[a] Values are expressed as the average ± standard deviation

and sweets and desserts were 1.1 ± 2.2 cups/day and 2.7 ± 3.4 servings/day, respectively. Mean meat (all kinds) and processed meat were 1.4 ± 1.1 and 0.4 ± 0.8 servings/day, respectively.

No significant differences were found between genders for all macronutrient, micronutrient, and food group intake, except for meat and processed meat intake which were found higher for men than women (Table 4).

The intake of iron, calcium, folic acid, vitamin B12, and vitamin B1 were found to be as under the DRI recommendations for 46, 48, 58, 14, and 34 % of the study population, respectively (Fig. 2).

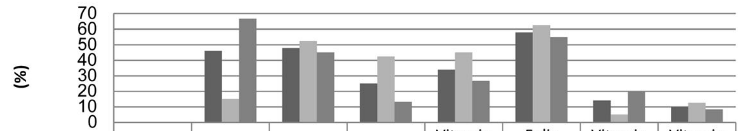
Table 4 Nutritional intake and comparison between men and women

Parameter[a]	All population	Men	Women	*P* value
Energy/day (kcal)	2,710.7 ± 1,275.7	2,615.6 ± 1,215.7	2,774.2 ± 1,320.4	0.545
Protein/day (gr)	114.2 ± 48.5	114.8 ± 43.3	113.8 ± 52.0	0.918
Protein (gr) per weight (kg)	1.0 ± 0.4	0.9 ± 0.3	1.0 ± 0.5	0.023
Fat/day (gr)	110.7 ± 54.5	106.3 ± 51.9	113.6 ± 56.4	0.511
Carbohydrates/day (gr)	321.6 ± 176.1	307.9 ± 170.7	330.7 ± 180.4	0.528
Fiber/day (gr)	32.1 ± 21.7	28.1 ± 16.5	34.8 ± 24.3	0.129
Calcium/day (mg)	1,182.4 ± 568.8	1,094.5 ± 450.3	1,240.9 ± 632.6	0.209
Iron/day (mg)	14.2 ± 7.3	13.3 ± 5.6	14.7 ± 8.3	0.325
Magnesium/day (mg)	435.7 ± 218.0	407.9 ± 182.3	454.3 ± 238.6	0.300
Phosphorus/day (mg)	1,799.3 ± 775.5	1,764.5 ± 713.5	1,822.6 ± 819.3	0.716
Potassium/day (mg)	4,125.0 ± 2,331.2	3,866.4 ± 1,847.7	4,297.4 ± 2,605.2	0.368
Sodium/day (mg)	4,664.8 ± 2,508.9	4,463.4 ± 2,078.7	4,800.0 ± 2,767.7	0.515
Zinc/day (mg)	13.2 ± 5.7	13.1 ± 5.0	13.2 ± 6.2	0.952
Copper/day (mg)	1.9 ± 1.0	1.8 ± 0.9	2.0 ± 1.1	0.458
Vitamin A/day (IU)	10,169.8 ± 9,941.4	8,320.5 ± 6,019.9	11,402.7 ± 11,748.1	0.089
Vitamin E/day (mg)	13.2 ± 7.6	12.2 ± 6.5	13.9 ± 8.2	0.272
Vitamin C/day (mg)	337.9 ± 299.5	315.8 ± 278.9	352.56 ± 313.9	0.550
Vitamin B1/day (mg)	1.6 ± 0.8	1.5 ± 0.8	1.6 ± 0.9	0.638
Vitamin B2/day (mg)	2.7 ± 1.3	2.5 ± 1.0	2.7 ± 1.4	0.445
Vitamin B3/day (mg)	21.4 ± 10.2	20.8 ± 8.6	21.8 ± 11.2	0.633
Vitamin B6/day (mg)	2.5 ± 1.4	2.4 ± 1.1	2.6 ± 1.6	0.408
Folic acid/day (μg)	421.5 ± 265.2	382.6 ± 172.9	447.4 ± 310.8	0.233
Vitamin B12/day (μg)	4.6 ± 2.2	4.5 ± 1.6	4.6 ± 2.5	0.798
Cholesterol/day (mg)	375.3 ± 215.6	386.1 ± 223.3	368.1 ± 212.0	0.684
Saturate fat acid/day (gr)	35.0 ± 17.3	33.5 ± 15.7	36.0 ± 18.3	0.473
MUFA (gr)	40.2 ± 20.7	38.2 ± 18.6	41.5 ± 22.1	0.439
PUFA (gr)	24.3 ± 14.3	24.0 ± 14.7	24.5 ± 14.0	0.854
Fructose (gr)	39.7 ± 33.9	41.2 ± 36.6	38.6 ± 32.2	0.707
DHA (gr)	0.10 ± 0.06	0.10 ± 0.05	0.10 ± 0.07	0.880
EPA (gr)	0.03 ± 0.03	0.03 ± 0.02	0.03 ± 0.03	0.379
Meat (all kinds)/day[b]	1.4 ± 1.1	1.8 ± 1.3	1.2 ± 0.9	0.012
Processed meat/day[b]	0.5 ± 0.8	0.7 ± 1.1	0.3 ± 0.4	0.053
Fish/day[b]	0.4 ± 0.4	0.3 ± 0.4	0.4 ± 0.5	0.666
Vegetables/day[b]	3.2 ± 2.2	1.8 ± 2.8	2.5 ± 3.5	0.071
Fruits/day[b]	1.6 ± 1.4	1.8 ± 1.7	1.5 ± 1.2	0.430
Sweets and desserts/day[b]	2.7 ± 3.4	2.1 ± 2.4	3.0 ± 3.9	0.133
Soft drinks (cups)/day	1.1 ± 2.2	1.5 ± 2.3	0.9 ± 2.2	0.201

IU international unit, *MUFA* monounsaturated fatty acid, *PUFA* polyunsaturated fatty acid, *DHA* docosahexaenoic acid, *EPA* eicosapentaenoic acid

[a] Values are expressed as the average ± standard deviation

[b] Serving/day



	Iron/day (mg)	Calcium/ day (mg)	Zinc/day (mg)	Vitamin B1/day (mg)	Folic acid/day (μg)	Vitamin B12/day (μg)	Vitamin C/day (mg)
All population(%)	46	48	25	34	58	14	10
Men(%)	15	52.5	42.5	45	62.5	5	12.5
Women(%)	66.6	45	13.3	26.7	55	20	8.3

Fig. 2 Prevalence of micronutrient intake below the DRI recommendations [14, 15]

Nutritional Deficiencies by Dietary Supplement Intake

The majority (59 %) of the study population undertook dietary supplementation, with similar distribution across gender except for lower vitamin D supplementation among men (32.5 vs. 58.3 % for men and women, $p = 0.011$, respectively) (Table 2). Mean vitamin B12 and folic acid levels were significantly higher in users of dietary supplementation compared to non-users (448.8 vs. 376.3 pg/ml, $p = 0.021$, and 9.9 vs. 8.1 ng/ml, $p = 0.031$, respectively). No other differences in nutritional status were noted between supplement users and non-users.

Discussion

Two main causes for nutrient deficiencies following BS are pre-operative deficiencies and inappropriate eating behavior, favoring foods with high-energy density and poor micronutrient content [10]. There is limited information available regarding dietary intake by obese patients prior to BS. The current study shows that most micronutrient intake did not reach to the DRI recommendations, despite high-caloric and macronutrient intake pre-operatively, which point to a consumption of poor quality diet low in micronutrients. Few previous studies reported also on low consumption of micronutrients in the diet before BS [19–21].

In our study, average energy intake was higher than the recommended caloric intake by age and gender of the 2010 American dietary guidelines for both genders [22], but similar to studies in Chilean women and a Spanish population, seeking BS [20, 21], and higher in 500 kcal/day than another study in 355 Spanish patients prior to BS [19].

The analysis of macronutrient intake in our population shows that energy obtained from fat intake (36 % of calories) was higher compared to the DRI recommendations (20–35 %), while energy obtained from carbohydrate intake (47 % of calories) was in the lower limits of the DRI recommendations (45–60 %) [17]. This macronutrient distribution is more typical to the Mediterranean diet pattern. However, our population consumed more sugar-sweetened beverages and sweets and desserts than the WHO's current recommendation for sugar consumption [23], more than double of the WHO's current recommendation for sodium consumption [24], and more processed meat than the World Cancer Research Fund public health recommendations [25], which is more reminiscent of a Western diet pattern. This diet may have negative effects on health, specifically on the risk for NAFLD, obesity, metabolic syndrome, type 2 diabetes, cardiovascular disease, and cancer [26].

Overall, we found a low prevalence of pre-operative nutritional deficiencies. Our findings are in contrast to other studies demonstrating more pronounced vitamin deficiencies among morbidly obese patients. However, vitamin D was found with high-deficiency prevalence, similar to previous studies [5, 7–9, 27–33].

Reasons discussed for high prevalence of vitamin D deficiency among morbidly obese patients were inadequate intake, reduced sun exposure, and decreased bioavailability of vitamin D due to it being deposited in adipose tissue [10]. There is no consensus defining optimal 25-hydroxyvitamin D concentrations. Growing evidence suggests that levels > 30 ng/ml may be sufficient to maintain health [6].

We examined deficiencies for vitamin B12 with the laboratory cutoff (<175 pg/ml) and also acut off <350 pg/ml. Laboratory cutoff values do not rule out the diagnosis of vitamin B12 deficiency in patients with compatible clinical abnormalities. However, serum vitamin B12 with the cutoff 350 pg/ml has sensitivity of 90 % and specificity of 25 % for detecting elevated level of methylmalonic acid, which is a more accurate marker of clinical vitamin B12 deficiency [34]. Further studies should test this cutoff for BS patients.

Hemoglobin level below normal range was found in just six patients, two of them with the genetic trait of Thalassemia minor known to affect hemoglobin levels [35]. The main causes of anemia are deficiencies in iron, vitamin B12, and folate [6], which were found to be with low prevalence in our population study. Those results are supported by a few studies [27, 30, 33] but in contrast to other studies that show higher anemia prevalence [8, 28, 29].

There are several explanations for the lower frequency of micronutrient deficiencies seen in our study as compared to previously reported data.

Overall, 59 % of our study population reported taking supplements at the baseline measurements, but unfortunately, we lack data regarding the exact type and duration of supplement used.

There is a lack of data on supplement consumption prior to BS, and few studies have shown that only 1–2 % of patients received supplements pre-surgery [20, 36]. Although we did not assess adherence to supplementation recommendations, we observed higher levels of folic acid and B12 levels in participants that reported the use of supplementation. Thus, we suggest a possible positive effect of pre-operative supplementation on the prevention of nutritional deficiencies. We assume the participants' commensurate high socioeconomic status can be related to high adherence for supplements prior to the surgery. Furthermore, this cross-sectional study is part of a RCT. Those trials are frequently performed in a highly motivated population of patients with high adherence rates to treatment [37]. We suggest that accurate assessment of adherence is crucial and relevant in understanding the true effectiveness of supplements and should be part of future research [38].

Our study has several limitations. First, it had a relatively small sample size; however, the numbers are similar to previously reported studies. Secondly, we did not measure some important micronutrients such as zinc, selenium, copper, and vitamin C. It is important to note that these micronutrients are hard to measure and expensive, making them unlikely to become a routine part of clinical routines. Thirdly, the study may suffer from selection bias because the participants allocated to LSG and not to malabsorptive procedure; they underwent the surgery in a private hospital and are participants of a RCT. They were of high socioeconomic status and may have been more compliant to supplement treatment. However, to the best of our knowledge, they represent similar populations worldwide, especially in Western countries.

In summary, in our study population, relatively low prevalence of vitamin and mineral deficiencies were found prior to surgery except for vitamin D. Most micronutrient intake did not reach to the DRI recommendations, despite high-caloric and macronutrient intake pre-operatively, which point to poor quality diet low in micronutrients. Possible explanations to our results might be high supplementation pre-operatively and high adherence to supplement treatment. We recommend a routine pre-operative screening and early supplementation if needed and application of a pre-operative program to optimize the eating pattern.

Compliance with Ethical Standard

Conflict of Interest Authors declare that they have no conflict of interest. Our study received grant from The Israeli Ministry of Health "Food and nutrition with implications on public health" (grant number 3–10470).

Ethical Approval All procedures performed in this study were approved by the institutional research committees in both participating hospitals and in accordance with the ethical standards of the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was pre-registered in the NIH registration website (TRIAL no. NCT01922830).

Statement of Informed Consent Informed consent was obtained from all individual participants included in the study.

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