

Comparison of Virtual Nutri Plus[®] and Dietpro 5i[®] software systems for the assessment of nutrient intake before and after Roux-en-Y gastric bypass

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OBJECTIVES: The assessment of nutritional intake before and after bariatric surgery assists in identifying eating disorders, nutritional deficiencies and weight loss/maintenance. The 7-day record is the gold standard for such an assessment and is interpreted using specialized software. This study sought to compare the Virtual Nutri Plus[®] and Dietpro 5i[®] software systems in assessing nutrient intake in obese patients with type 2 diabetes mellitus who underwent a Roux-en-Y gastric bypass.

METHODS: Nutritional intake was assessed in 10 obese women with type 2 diabetes mellitus before and 3 months after Roux-en-Y gastric bypass. The 7-day record was used to assess food intake and then, the Virtual Nutri Plus[®] and Dietpro 5i[®] software systems were used to calculate calorie, macronutrient and micronutrient intake based on validated food chemical composition databases. Clinicaltrials.gov: NCT01251016.

RESULTS: During the preoperative period, deficits in the ingestion of total fiber and 15 out of 22 estimated micronutrients were observed when using the Virtual Nutri Plus[®], compared to deficiencies in total fiber and 4 micronutrients when using the Dietpro 5i[®]. During the postoperative period, both the Virtual Nutri Plus[®] and Dietpro 5i[®] systems detected deficits in the ingestion of total fiber, carbohydrates and 19 micronutrients, but only the Virtual Nutri Plus[®] detected deficits in complex B vitamins (except B12) and minerals.

CONCLUSION: Virtual Nutri Plus[®] was more sensitive than Dietpro 5i[®] for the identification of deficits in nutrient intake in obese, type 2 diabetes mellitus patients undergoing Roux-en-Y gastric bypass.

KEYWORDS: Diseases; Health Care; Information Science.

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INTRODUCTION

Obesity is a global health problem that is associated with numerous life-threatening and disabling comorbidities, such as hypertension, nonalcoholic steatohepatitis, type 2 diabetes mellitus (T2DM) and cardiac arrest (1,2). Currently, bariatric surgery is accepted as the most effective treatment for morbidly obese patients (body mass index [BMI] of 35 kg/m² associated with two or more comorbidities or BMI ≥40 kg/m²) to allow weight loss and to improve or

alleviate obesity-associated comorbidities (1,3). Restricted food intake and/or nutrient malabsorption after bariatric surgery are important for the long-term control of body weight, but they can also aggravate previous nutritional deficits and contribute to the onset of new nutritional deficiencies (4).

According to the most recent Brazilian survey (2008-2009), Brazil has an obesity incidence of 12.4% for men and 16.9% for women (5). The survey from 2008-2009 lacks data for morbidly obese individuals, but a previous Brazilian survey (2002-2003) found the rate of severely obese Brazilians to be 0.64% (6).

The estimation of nutrient intake is important for the careful monitoring of nutritional intake after bariatric surgery. The 7-day record is considered to be the gold standard tool for the assessment of food intake, as it provides detailed information about the pattern of food ingestion (7). Several software systems have been developed to calculate the data collected by this instrument in terms of

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nutrients, thereby allowing the estimation of nutrient intake (7).

Two different software systems for the assessment of nutrient intake have been developed in Brazil and are extensively applied in local clinical practice (7). Because these systems vary in the aspects of nutrients they are designed to measure, they may provide different evaluation outcomes. Our study compared the Virtual Nutri Plus® (VNP) and Dietpro 5i® (DP) software systems to provide nutritional information of obese patients with T2DM before and after Roux-en-Y gastric bypass (RYGB).

METHODS

Ethical considerations

The current study is part of a larger clinical trial that aims to analyze different aspects involved in the reversion of T2DM after RYGB and the current study is registered at Clinicaltrials.gov: NCT01251016. This study was performed according to the ethical recommendations of the Declaration of Helsinki and the Ethical Committee of the Faculdade de Medicina da Universidade de São Paulo. All patients enrolled in the study provided written informed consent.

Patients

Ten adult (18-60 years old) obese patients (BMI > 35 kg/m²) with a proven diagnosis of T2DM (fasting plasma glucose - FPG > 126 mg/dL and HbA1c > 7%) and who used oral antidiabetic medication participated in the study. All patients were admitted to the Surgical Division of the Gastroenterology Department of the Hospital das Clínicas da Faculdade de Medicina da Universidade de São Paulo (ICHC-FMUSP) between April 2010 and December 2012 for elective RYGB. The RYGB procedure was standardized as simply gastric by-pass (8). Exclusionary criteria included insulin use, the diagnosis of thyroid disease and any participation in other clinical trials. All patients were treated for *Helicobacter pylori* bacterial infection prior to surgery.

Nutritional assessment

Nutritional assessment was performed 1 month before and 3 months after RYGB. Food intake was determined using the 7-day record (5). For the 7-day record, the type and amount of food and beverages consumed were self-recorded by the patient for 7 consecutive days, including one weekend, after patients were trained during a session with two trained nutritionists (MMS and PCS). The amount of food consumed was recorded in terms of cooking units (spoons, cups, etc.) using the *Livro Consumo Alimentar - Visualizando Porções* (Food Consumption book - Viewing Portions) (9). During the interview with the nutritionists (average time of 90 minutes), the patient was instructed and trained to use this book, as well as how to recognize and record the foods consumed correctly. All patients took the book home to register the 7-day food record as they had been instructed. In addition, the dieticians called each patient every 2 days to monitor the self-recording process. All of the patients' diaries were read by MMS and were individually discussed if needed to verify accuracy. Adherence of the patients to the self-recording program was 100%. The data obtained were double-registered in a computerized database and used to calculate the consumption of total calories, macronutrients and

micronutrients using the VNP (10) and DP (11) software programs.

The nutritional data studied were as follows: total calories; protein; carbohydrates; total, saturated, monounsaturated and polyunsaturated fats; cholesterol; total, soluble and insoluble fiber; vitamins A, B1, B2, B3, B5, B6, B12, C, D and E; calcium, copper, folate, iodine, iron, magnesium, manganese, phosphorus, potassium, selenium, sodium and zinc. Vitamins B1, B2, B3, B5 and B6, as well as zinc, copper, folate, iodine, phosphate, manganese, potassium and selenium were only estimated by VNP, as DP does not assess these micronutrients. The following data sources were used to determine the food chemical composition: the food chemical composition table developed by Sonia Tucunduva Philippi (12) and the Brazilian food chemical composition table, TACO (13). Pre and postoperative nutritional consumption were analyzed using the daily recommendations for nutrient intake of the Dietary Reference Intakes (DRIs) of the Food and Nutrition Board from the Institute of Medicine (14).

Statistical analysis

Sample homogeneity was tested for all variables using the Kolmogorov-Smirnov test. Comparisons between pre and postoperative periods were evaluated using the paired t-test or Mann-Whitney test. A significance level of 5% was considered for all tests, which were performed using the R program (version 3.0.2) (15).

RESULTS

Patients

All of the included patients were women aged 32-54 years and their descriptive data are provided in Table 1.

Nutritional deficiencies before and after bariatric surgery

General nutritional data were obtained before and 3 months after bariatric surgery using the 7-day record for data food collection and the VNP and DP software programs for the estimation of nutritional content (Table 2). Based on the nutritional recommendations from DRIs, preoperative deficits in nutrient intake were observed for total fiber, vitamin D, calcium, magnesium and zinc by both the VNP and DP software programs. Deficits in vitamins A, B5, B6, E and deficient intake of iodine, folate, manganese, phosphorus, potassium and selenium were identified only with the VNP software. All of the deficits in nutritional ingestion identified before RYGB were maintained postoperatively, as shown by both the VNP and DP software programs. Only carbohydrate and iron

Table 1 - Descriptive data (means \pm standard deviation) of obese women with T2DM (n = 10) before and 3 months after RYGB.

Variable	Mean
Age	46.5 \pm 6.6
Height	1.6 \pm 0.1
Body weight	114.8 \pm 15.0
Body mass index	45.7 \pm 4.1
Waist circumference	128.4 \pm 13.4
Resting energy expenditure (Kcal)	203 \pm 241.2
Resting energy expenditure per kilogram	17.8 \pm 2.2



Table 2 - General nutritional data (means \pm standard deviation) of obese women with T2DM (n = 10) obtained before and 3 months after RYGB using the 7-day record to determine food intake and either the VNP or DP program to estimate the nutritional content.

Variable	Recommended values ¹¹	Preoperative		Postoperative	
		Virtual Nutri®	Diet Pro®	Virtual Nutri®	Diet Pro®
CAL	-	1519.1 \pm 291.5	1626.8 \pm 318.0	995.3 \pm 276.5	1093.7 \pm 297.6
PTN	46.0	63.7 \pm 10.4	60.2 \pm 10.15	49.9 \pm 17.3	48.7 \pm 16.5
CHO	130.0	199.9 \pm 35.1	205.5 \pm 40.8	118.6 \pm 32.6	122.9 \pm 32.4
TFAT	-	54.8 \pm 14.8	67.0 \pm 18.3	37.6 \pm 10.6	46.5 \pm 12.5
SFAT	-	16.4 \pm 4.9	12.1 \pm 4.8	10.8 \pm 3.7	9.0 \pm 3.5
MFAT	-	14.7 \pm 3.9	12.6 \pm 5.1	9.6 \pm 3.3	8.6 \pm 2.9
PFAT	-	11.7 \pm 2.9	10.7 \pm 3.1	7.9 \pm 1.7	7.9 \pm 1.5
CHOL	-	173.5 \pm 51.4	154.2 \pm 45.7	124.2 \pm 57.2	116.9 \pm 53.3
TFIB	21.0	16.8 \pm 6.6	12.9 \pm 4.0	10.1 \pm 3.7	8.6 \pm 2.7
SFIB	-	1.9 \pm 0.7	3.0 \pm 1.9	1.3 \pm 0.8	2.0 \pm 1.0
IFIB	-	3.5 \pm 1.4	4.0 \pm 1.8	2.3 \pm 1.0	2.6 \pm 1.2
VITA	700.0	645.3 \pm 684.7	873.9 \pm 874.6	520.4 \pm 605.8	559.3 \pm 331.2
VITB1	1.1	2.0 \pm 1.7	NA	0.9 \pm 0.5	NA
VITB2	1.1	0.9 \pm 0.3	NA	0.7 \pm 0.4	NA
VITB3	14.0	15.1 \pm 4.2	NA	8.6 \pm 3.0	NA
VITB5	5.0	1.9 \pm 0.5	NA	1.7 \pm 0.7	NA
VITB6	1.5	1.0 \pm 0.3	NA	0.8 \pm 0.3	NA
VITB12	2.4	2.9 \pm 4.9	3.8 \pm .0	3.7 \pm 6.6	4.4 \pm 6.3
VITC	75.0	467.0 \pm 1094.7	302.7 \pm 461.9	134.0 \pm 112.6	124.1 \pm 72.0
VITD	15.0	4.5 \pm 11.6	5.8 \pm 11.5	0.5 \pm 0.4	2.4 \pm 1.2
VITE	15.0	8.8 \pm 2.4	17.7 \pm 2.8	6.2 \pm 2.1	12.7 \pm 2.3
CALC	1200.0	535.8 \pm 166.1	580.1 \pm 213.1	578.1 \pm 214.2	630.9 \pm 269.4
COPP	0.9	6.7 \pm 18.9	NA	0.5 \pm 0.3	NA
FOL	400.0	134.6 \pm 37.3	NA	92.9 \pm 41.3	NA
IOD	150.0	16.9 \pm 14.5	NA	26.3 \pm 15.9	NA
IRO	8.0	8.9 \pm 1.3	10.2 \pm 1.2	6.0 \pm 2.1	7.0 \pm 3.1
MAGN	320.0	153.0 \pm 32.0	166.6 \pm 32.6	101.2 \pm 32.5	128.2 \pm 37.04
MANG	1.8	1.3 \pm 0.4	NA	0.8 \pm 0.2	NA
PHOS	700.0	631.0 \pm 175.3	NA	444.1 \pm 158.6	NA
POT	4.7	1.7 \pm 0.4	NA	1.2 \pm 0.4	NA
SEL	55.0	45.9 \pm 10.3	NA	26.6 \pm 15.8	NA
SOD	1.3	3.1 \pm 0.4	2.5 \pm 0.5	2.5 \pm 0.5	1.8 \pm 0.3
ZIN	8.0	6.5 \pm 1.8	5.6 \pm 1.6	5.0 \pm 2.2	5.3 \pm 2.1

CAL: calories (Kcal); PTN: protein (g); CHO: carbohydrates (g); TFAT: total fat (g); SFAT: saturated fat (g); MFAT: monounsaturated fat (g); PFAT: polyunsaturated fat (g); CHOL: cholesterol (mg); TFIB: total fiber (g); SFIB: soluble fiber (g); IFIB: insoluble fiber (g); VITA: vitamin A (RE); VITB1: vitamin B1 (mg); VITB2: vitamin B2 (mg); VITB3: vitamin B3 (mg); VITB6: vitamin B6 (mg); VITB12: vitamin B12 (μ g); VITC: vitamin C (mg); VITD: vitamin D (μ g); VITE: vitamin E (mg); CALC: calcium (mg); COPP: copper (mg); FOL: folate (μ g); IOD: iodine (μ g); IRO: iron (mg); MAGN: magnesium (mg); MANG: manganese (mg); PHOS: phosphorus (mg); POT: potassium (g); SEL: selenium (μ g); SOD: sodium (g); ZIN: zinc (mg).

levels demonstrated new nutritional intake deficits during the postoperative period, as identified by both software systems and vitamins B1 and B3 and copper were also identified with the VNP software.

Changes in nutritional intake before and after RYGB

Energy and macronutrients. Based on the nutritional recommendations from DRIs, decreased ingestion of calories and macronutrients (carbohydrates, total fat and fiber) was observed with both the VNP and DP programs (Figure 1). No differences were found for protein consumption by either software program ($p \geq 0.05$).

Subclasses of macronutrients. Based on changes in food intake, the decreased ingestion of monounsaturated and polyunsaturated fats, as well as insoluble fibers, were observed with the VNP software, but only differences in polyunsaturated fat were uncovered using the DP software (Figure 2). No significant changes were found for cholesterol consumption using any of the studied tools ($p \geq 0.05$).

Vitamins

According to the change in food intake, the decreased ingestion of vitamin E was observed with both the VNP and DP programs (Figure 3). Vitamin B1 and B3 levels are only calculated by the VNP program, which showed that there was a decreased level of intake of these vitamins in the postoperative period ($p = 0.002$ and $p = 0.004$, respectively). No significant changes were found for the levels of consumed vitamins A, B2, B5, B6, B12, C and D using any of the studied tools ($p \geq 0.05$).

Minerals

Based on the change in food intake, decreased levels of the ingestion of iron, sodium and magnesium were observed with both the VNP and DP programs (Figure 3). Copper, folate, phosphorus, manganese, potassium and selenium are only calculated for the VNP software and decreased levels of ingestion of these minerals were found ($p \leq 0.01$). No significant changes were found for calcium, iodine or zinc consumption by any of the studied tools ($p \geq 0.05$).

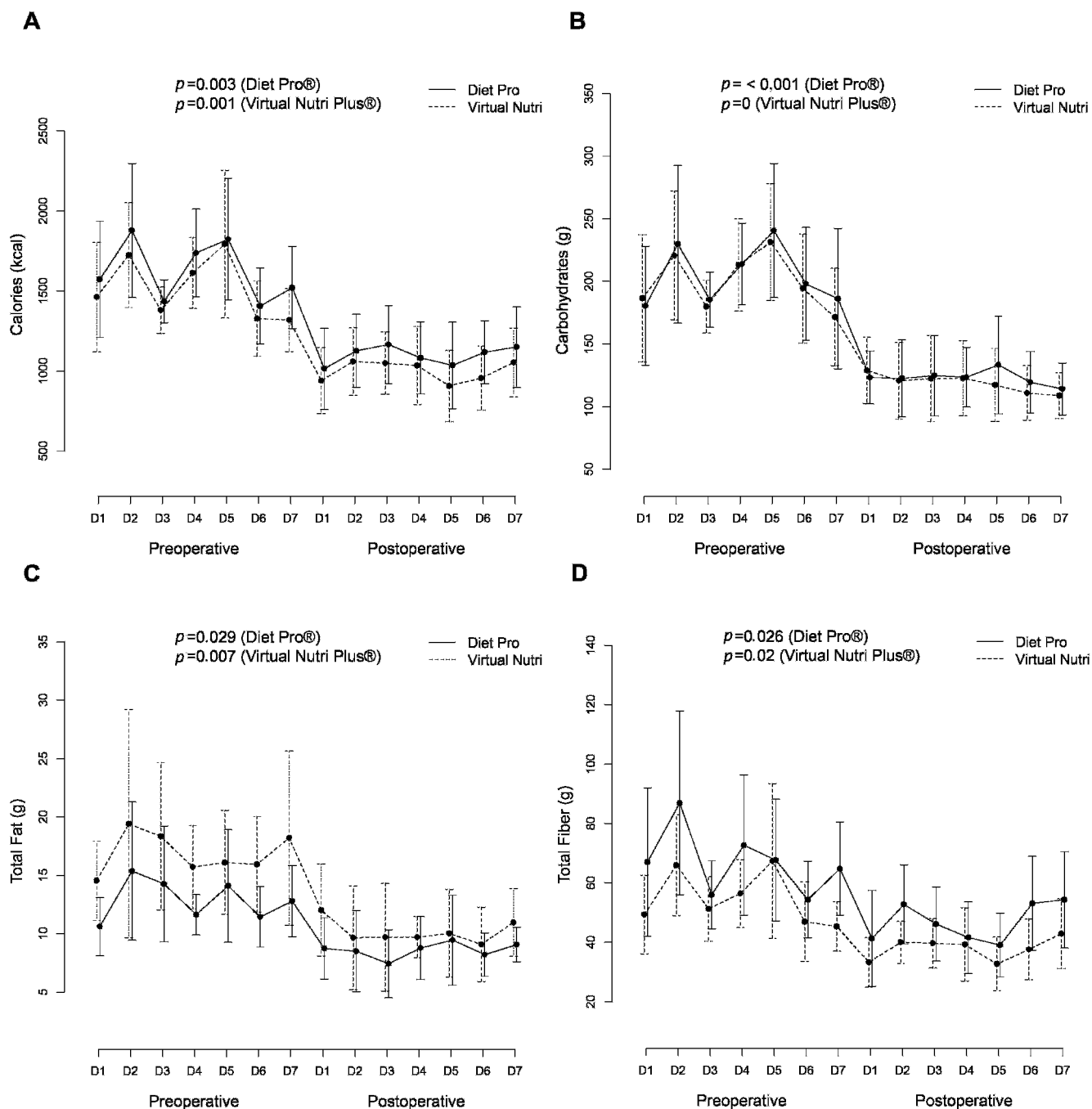


Figure 1 - Changes in the amount of calories and total macronutrients ingested by obese women with type 2 diabetes mellitus ($n = 10$) before and 3 months after a Roux-en Y gastric bypass, as calculated using 7-day records to evaluate what was eaten and both the Virtual Nutri® and Dietpro 5i® software systems to estimate the nutritional content. Changes in the intake of: (A) calories, (B) carbohydrates, (C) total fat and (D) total fiber.

DISCUSSION

Despite the benefits of weight loss and the alleviation of obesity-associated comorbidities observed after RYGB, this procedure may increase previous nutritional deficiencies and other nutritional deficiencies can appear due to the limitation of the size of the food reservoir and malabsorption (4,8). Considering the relevance of identifying deficits in the intake of nutrients before and after bariatric surgery, our study compared two different available nutritional

software systems to estimate nutrient ingestion. Our data showed that these instruments could distinctly identify deficits in nutrient intake in obese T2DM patients who underwent RYGB.

The VNP software was developed by Philippi et al. (10) for both clinical and academic use and allows for the calculation of the nutritional value of a meal based on a food data source composed of 1,711 foods and 2,020 variations of these foods (natural, preparations and industrialized). DP software was developed by the Dietpro team for clinical use

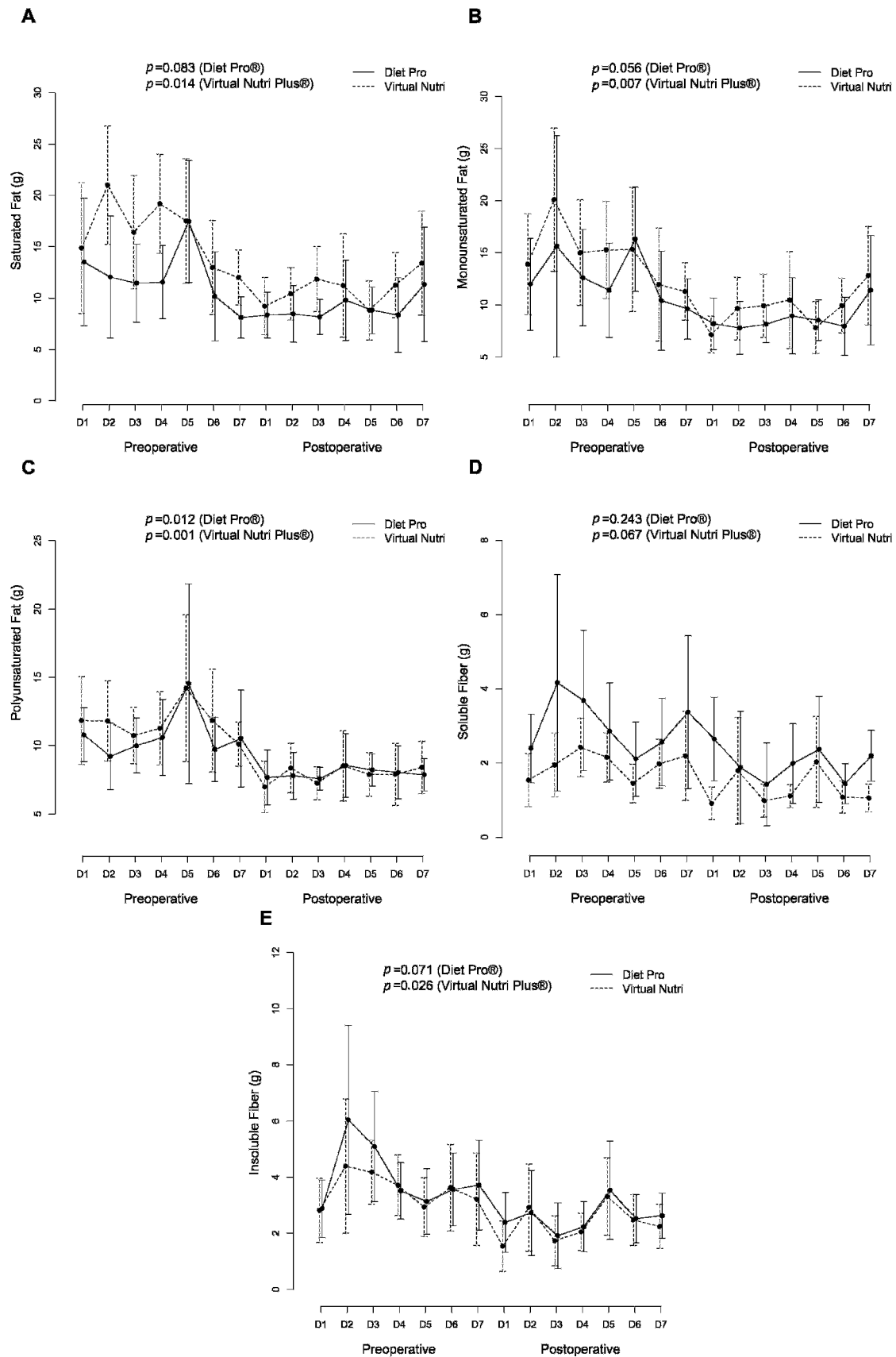


Figure 2 - Changes in the subcategories of fat and fiber ingested by obese women with type 2 diabetes mellitus (n = 10) before and 3 months after a Roux-en Y gastric bypass. Changes in the intake of: (A) saturated fat, (B) monounsaturated fat, (C) polyunsaturated fat and (D) soluble fiber and (E) insoluble fiber.



and allows for the calculation of the nutritional value of a meal and it can also be compared with five different validated databases of dietetic recommendations (RDA, SBAN, AMDRs, OMS 2003 and DRI). Both software systems provide different tools to improve the estimation of the amount of food eaten. These are standardized measures, but as mentioned before, we presently use the Food Consumption book - Viewing Portions (9) to standardize the method for nutrient estimation using these different software systems. Additionally, the food chemical composition table, TACO (13), was used for nutritional estimation in the Dietpro system whenever the food component to be registered was missing in the software database.

According to our data and based on the nutritional recommendations from DRIs, during the preoperative period, both the DP and VNP nutritional software systems could identify deficiencies in the ingestion of total fiber, vitamin D, calcium, magnesium and zinc. Accordingly, the decreased consumption of fruits and vegetables and increased consumption of high-fat diets and sweetened beverages by obese individuals are associated with decreased levels of fiber, vitamins A, C and D, calcium and folate ingestion (16-18). It is important to highlight that although these deficiencies were identified with both the DP and VNP software programs, only VNP could identify deficits in the ingestion of other micronutrients, such as vitamin A and vitamin E.

Among the deficits detected only by VNP during the preoperative period were deficits in vitamins A and E. In another study, deficiencies in vitamin A were found in 11% of obese patients before bariatric surgery and low vitamin E levels were also reported (19). Moreover, using nationally representative cross-sectional data from the National Health and Nutrition Examination Survey III (NHANES III), Kimmons et al. reported that overweight men had a lower chance of low vitamin E status than normal-weight men. Furthermore, low levels of selenium and selenium deficiency were found in 58% of morbidly obese patients before bariatric surgery (19).

Vitamins A and E have been found to be inversely correlated with oxidative stress, insulin resistance, impaired glucose metabolism, cancers, and age-related macular degeneration and are involved in low-density lipoprotein (LDL) protection against oxidation, thereby contributing to the prevention of atherosclerosis (20). Selenium is important for normal thyroid functions and immunological reactions and is involved in antioxidative reactions, thus potentially contributing to the prevention of chronic diseases (21). Considering the importance of adequately identifying changes in these micronutrients, in terms of preoperative replenishment, our preoperative data suggest that VNP software may be a better approach than DP to recognize deficits in nutritional ingestion in obese patients.

Several micronutrient deficiencies have been reported after RYGB procedures, including vitamins A and D and folate (22,23). According to our data, the VNP software was able to show postoperative deficiencies in the consumption of all of these micronutrients, as well as an increased deficit in folate intake. On the other hand, DP software failed to show preoperative deficiencies in vitamin A ingestion.

In addition, the VNP software allowed for the identification of postoperative deficiencies in vitamin B1 and copper intake, while the DP software was unable to calculate the vitamin B1 and copper intake. This may be a disadvantage

of the DP software for assessing nutrient intake after RYGB, as vitamin B1 and copper deficiencies are quite common following RYGB. In particular, there is biochemical evidence for a relationship between vitamin B1 and thiamine deficiency in up to 49% of patients (24). Vitamin B1 deficiency, which can lead to symptoms of beriberi, is a major nutritional complication. Additionally, low serum copper levels in susceptible individuals can lead to anemia, neutropenia and pancytopenia (25).

By detecting changes between the pre and postoperative periods, the VNP and DP software systems identified similar significant deficits in energy and total macronutrient ingestion. However, the VNP software highlighted a decrease in all of the subclasses of the macronutrients studied, while the DP system only demonstrated deficits in the polyunsaturated fatty acids. The mean energy values observed with the VNP software were in accordance with those reported in the literature (mean 1,000 kcal/day of calories daily ingestion at 3 and 12 months postoperatively) (26-28). In addition, Rocha et al. also observed reduced intakes of calories, carbohydrates and fats after RYGB using a 3-day record tool (29).

The ingestion of total fiber was found to be below the daily recommended level during the preoperative period in our study and this deficiency was aggravated postoperatively, as shown by both the VNP and DP software systems. Our data agree with those of Novais et al. (30), who found that after RYGB, women had a very low level of fiber ingestion, although the values differed depending on the nutritional tools applied. The authors used two separate 24 h food recalls, including one weekend, to assess total fiber ingestion, and the Nutwin® software was used to estimate intake (30). One possible reason for these observations may be related to the fact that the authors evaluated a longer postoperative time period, ≥ 2 years, than in our study.

Another important finding of our study was that the VNP software was more sensitive than the DP software for identifying significant changes in micronutrient ingestion between pre and postoperative periods, according to the nutritional recommendations from DRIs. This would be expected, as DP software does not calculate 12 of the micronutrients evaluated in our study. Of note, eight (vitamins B1 and B3, copper, folate, manganese, phosphorus, potassium and selenium) of the 12 micronutrients that were not evaluated with the DP software showed decreased levels of intake between the pre and postoperative periods, as identified by the VNP software. All of these deficiencies in micronutrient intake may lead to serious clinical complications.

Deficiencies in vitamins B1 and selenium, which were not calculated by the DP software, have also been previously reported. Rossi et al. found a significant decrease in iron, zinc, vitamin B12 and vitamin B1 ingestion in women after RYGB using a 4-day record instrument (31) and Freeth et al. (32) found a significant decrease in selenium ingestion 3 months after RYGB using a 3-day record instrument.

Low biochemical levels of iron, vitamin B12, vitamin D and calcium are described as predominant after RYGB (33). In the present study, both software systems identified a reduction of intake in food containing iron by comparing pre and postoperative periods, but these systems failed to show changes in vitamin B12, vitamin D and calcium. However, the ingestion of vitamin D and calcium was

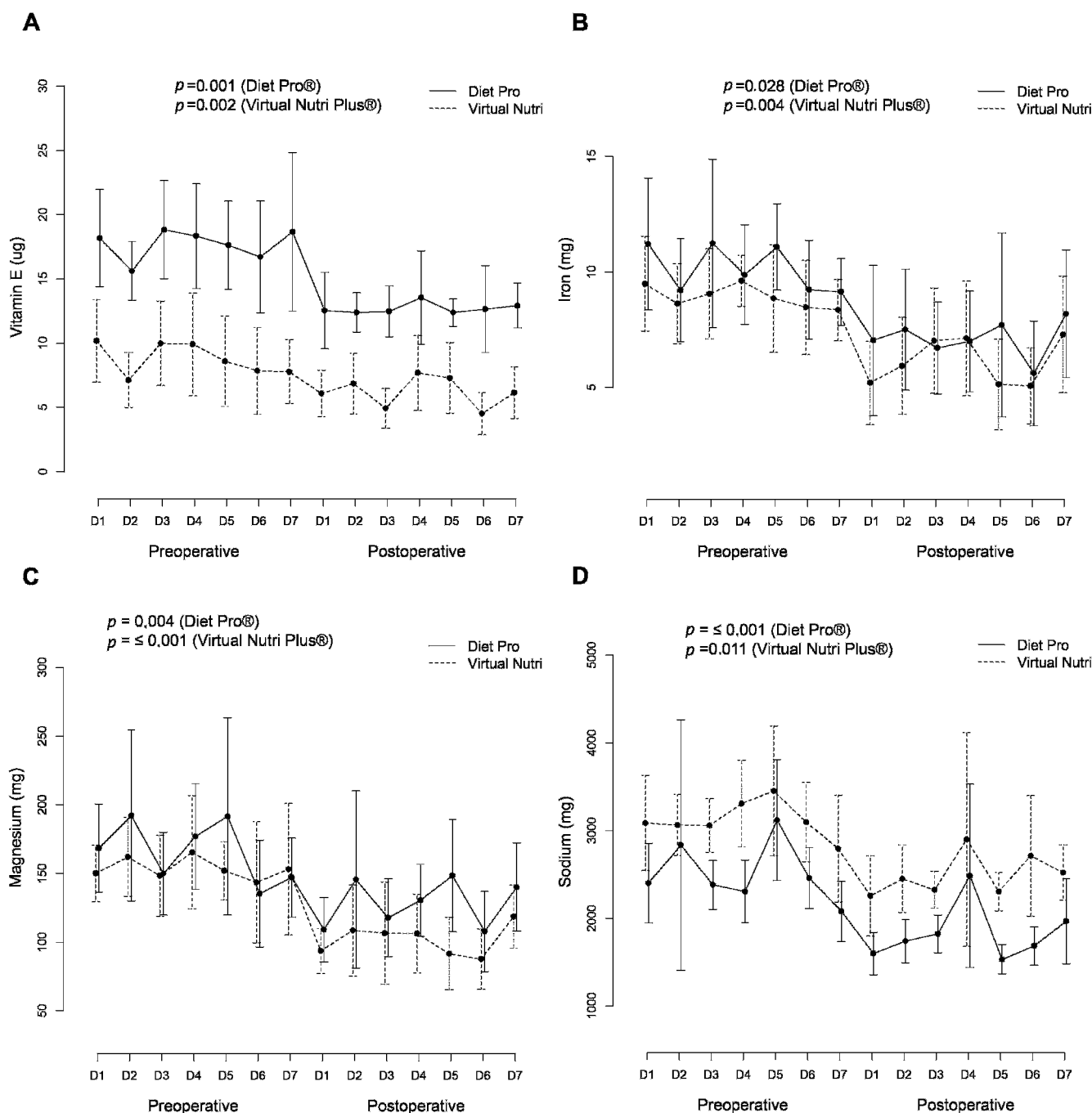


Figure 3 - Changes in the ingestion of micronutrients by obese women with type 2 diabetes mellitus (n = 10) before and 3 months after a Roux-en Y gastric bypass. Changes in: (A) vitamin E, (B) iron, (C) magnesium and (D) sodium.

already lower than the daily recommended level before surgery (as detected using both software systems) and remained lower than the daily recommended level during the postoperative period. This situation may have impaired the observation of significant decreases between these periods. Regarding vitamin B12, one of the patients ate fried beef liver, which is a very rich source of vitamin B12, during the postoperative period and this may have resulted in an overestimation of vitamin B12 consumption.

After bariatric surgery, protein is the major macronutrient associated with malnutrition (34). The guidelines for the perioperative nutritional, metabolic and nonsurgical

support of the bariatric surgery patient suggest that bariatric patients should ingest a minimum protein level of 60 g/day. Although neither VNP nor DP could detect deficiencies in protein ingestion by considering the DRI recommendations, both nutritional software programs demonstrated protein ingestion levels of less than 60 g/day, as recommended clinically in the postoperative period (35). One possible explanation for patients not presenting with severe deficiency in protein ingestion in the postoperative period may be because in our hospital, bariatric patients are instructed to consume protein before other macronutrients during a meal, due to early satiety.



Overall, both the VNP and DP software programs detected deficits in nutritional intake before and after RYGB that were consistent with those deficits found in the literature. However, these nutritional software systems differ mainly in terms of micronutrient estimation, as the DP program does not evaluate a significant number of micronutrients that are estimated by the VNP, which could lead to serious clinical complications in obese patients who undergo RYGB. In addition, when considering only the intake of micronutrients evaluated by the DP software, it failed to detect deficiencies in vitamin A and vitamin E intake during the preoperative period that were detected by VNP.

In conclusion, DP nutritional software was efficient for detecting intake deficits before and after RYGB with less sensitivity than VNP, mainly when comparing changes that occurred between these periods. On the other hand, the VNP software detected deficits in nutrient intake before and after RYGB, including several important micronutrients that could not be estimated by DP, which were consistent with those found in the literature. In conclusion, our data suggest that the VNP software program appears to be more sensitive and more comprehensive than the DP software for identifying significant deficits in micronutrient ingestion before and after RYGB, as well as for detecting decreases in micronutrient ingestion between these periods.

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AUTHOR CONTRIBUTIONS

Silva MM performed the nutritional evaluation of patients and participated in database management, analysis, interpretation and manuscript writing. Sala PC performed the enrollment and assignment of patients and participated in the study design, coordination and data interpretation. Cardinelli CS provided assistance with the softwares and reviewed the food diaries, tables and figures. Torrinhas RS participated in data interpretation and manuscript writing. Waitzberg DL conceived the study and participated in the study design, coordination and data interpretation. All authors critically reviewed the manuscript for important intellectual content and approved the final version to be published.

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