



# Preoperative Carbohydrate Quality Index Is Related to Markers of Glucose Metabolism 12 Months After Roux-en-Y Gastric Bypass

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## Abstract

**Objective** The role of carbohydrates in weight loss in patients undergoing bariatric surgery (BS) remains poorly understood. Therefore, this study aimed to verify the relationship of the carbohydrate quality index (CQI) with weight loss and cardio-metabolic risk markers up to 1 year after BS.

**Material and Methods** This study included 50 patients with obesity undergoing Roux-en-Y gastric bypass. Data collection was performed preoperatively and 3 and 12 months after surgery. The foods consumed were documented using a 24-h food recall in 3 days. The CQI was calculated considering the following parameters: dietary fiber intake, sugar level; whole grains: proportion of total grains; solid carbohydrate: total carbohydrate ratio.

**Results** From the total study sample, 58 participants were followed up for 3 months, and eight participants dropped out of the study. The remaining 50 patients were followed up for 12 months. Subjects were classified into tertiles according to the index score. A 1-unit increase in CQI was associated with a  $-1.02$  decrease in insulin concentrations at 12 months and a  $-1.04$  decrease in HOMA-IR. Concerning the total sample, the median of the CQI was 8 points and did not change at 3 and 12 months after surgery, but there was an improvement in some components of the index.

**Conclusion** The data suggest that the quality of carbohydrates can interfere with markers of insulin resistance after BS and the quality of carbohydrates is a point to be guided in patients undergoing BS.

**Keywords** Bariatric surgery · Carbohydrate quality index · Insulin · Gastric bypass · Macronutrients · Food intake

## Key Points

- Insulin was different between the thirds of the CQI.
- Increase in CQI at baseline is associated with decrease in insulin and HOMA-IR at 12 months.
- CQI was 8 points and did not change at 3 and 12 months after surgery.

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## Introduction

Bariatric surgery (BS) is the most effective treatment for patients with severe obesity owing to its long-lasting benefits and greater weight loss [1]. However, despite its multiple benefits, BS remains a challenge for patients with insufficient weight loss [2–4] and the risk of weight regain [2–5]. Thus, strategies are needed to improve the treatment of severe obesity, especially in the long-term.

In this context, a prospective cohort study analyzed the quality of carbohydrates in healthy adults using the carbohydrate quality index (CQI) [6]. The CQI assesses the quality of carbohydrates according to GI, fiber content, solid or liquid form, and degree of processing. The research findings suggested that poorer carbohydrate quality increased the risk of being overweight, obese, or gaining weight over time [6]. However, the role of carbohydrates in weight loss in patients undergoing BS remains poorly understood. Moreover, no studies have assessed the quality of carbohydrates through the CQI in post-bariatric patients. Therefore, this study

aimed to verify the relationship between CQI (at baseline) weight loss and cardiometabolic risk markers up to 1 year after RYGB.

## Casuistry and Methods

### Ethical Aspects

This study was conducted in accordance with the guidelines set out in the Declaration of Helsinki [7]; the study proposal and protocol were submitted to the Ethics and Research Committee of the Universidade Federal de Viçosa and approved under opinion No. 1.852.365. Before beginning data collection, all participants signed the Free and Informed Consent Form (FICT).

### Study Characterization and Sample Size

This prospective cohort study followed 50 patients for 12 months. The study initially evaluated 58 patients; however, 8 withdrew before 1 year of follow-up due to personal reasons. Only those who continued participation in the study for up to 12 months were included in the analyses. The inclusion criteria were males or females with severe (grade III) or grade II obesity with comorbidities who underwent BS. Surgery was performed using the Roux-en-Y gastric bypass (RYGB) technique for the first time by laparoscopy or exploratory laparotomy.

The sample size was calculated according to the methodology proposed by Pereira et al. (2014) [8]. The calculation considered the main study variables (weight, body mass index [BMI], and waist circumference) as outcomes and a power of 95%. Thus, 35 participants were calculated as being needed; accounting for a probable 40% of losses, and the final sample size was set at 50 participants.

### Data Collection

The same researcher, a registered and duly trained nutritionist (S.L.P) followed up with all patients. Data collection was performed preoperatively (baseline) and at 3 and 12 months postoperatively, from February 2017 to September 2018. Consultations were scheduled and held at the Nutrition Laboratory of the Universidade Federal do Tocantins, campus Palmas/Tocantins.

### Sociodemographic Information

Sociodemographic and economic data were collected using a semi-structured questionnaire through face-to-face interviews.

## Anthropometry and Body Composition

Body weight (kg) data were obtained using a digital electronic scale (Welmy®) with a capacity of 300 kg and a precision of 100 g. Height (m) was measured using a stadiometer fixed to a wall without a plinth, according to the recommendations of Jelliffe [9]. Subsequently, BMI was calculated, and anthropometric status was determined based on the WHO classification [10]. Weight-based measurements were also performed, excess body weight (EBW) = pre-weight (kg) – ideal weight (kg) was assessed with a BMI of 24.9 kg/m<sup>2</sup>. Waist circumference (WC) and neck circumference (NC) were evaluated using a flexible, inelastic 2-m-long tape. Waist circumference was measured using the method of Calaway et al. (1998) [11], while NC was measured according to the technique adopted by Ben-Noun et al. (2003) [12].

Body composition was measured using a tetrapolar bioelectrical impedance analyzer (model BIA 310 Biodynamics®) according to the manufacturer's protocol. Body fat (BF) was expressed as a percentage.

### Cardiometabolic Risk Markers

All biochemical analyses were performed by a private outsourced laboratory. Patients were instructed to fast for at least 8 h and at most 12 h before sample collection. Serum concentrations of glucose, triglycerides, total cholesterol, and fractions were determined using an enzymatic colorimetric test; insulin was measured using electrochemiluminescence immunoassay. Insulin resistance was assessed using the homeostatic model for insulin resistance (HOMA-IR) [13]. In addition, the presence of metabolic syndrome (MS) was measured according to International Diabetes Federation guidelines [14].

### Food Consumption

In the postoperative period, they followed a standard diet with consistency evolution according to the guideline [15]. Patients were instructed to prefer whole carbohydrates but no formal prescription was made. Food consumption was analyzed using a 24-h recall (R24); at each time point (baseline, 3, and 12 months) three R24 were collected. The R24 was typed and analyzed by the same researcher (S.L.P) using BrasilNutri® [16]. In addition, nutritional composition analyses of macronutrients, calories, and fiber were performed using the STATA® software version 13.0.

### Calculation of the Carbohydrate Quality Index

The CQI was calculated according to the methodology proposed in the SUN project article (*Seguimiento Universidad*

*de Navarra*) [17]. The following four criteria were considered to identify the CQI: the proportion of whole-grain carbohydrates to total grain carbohydrates (total grain = sum of carbohydrates from whole grains + refined grains + products prepared with refined flours), that is, whole-grain carbohydrates divided by the amount of total grain carbohydrates; GI (negatively weighted); the ratio of solid carbohydrates to total carbohydrates (solid + liquid) divided by the amount of solid carbohydrates; and total dietary fiber intake (g/day) (Table 1). Liquid carbohydrates were calculated by summing the consumption of sweetened beverages and fruit juice, whereas solid carbohydrates corresponded to the carbohydrate content of the remaining carbohydrate-containing foods [6]. The glycemic index of each food consumed was obtained from the University of Sydney database [18]. The weighted daily dietary GI was calculated following a standard protocol: weighted GI = initial GI (glycemic index of each food obtained from the database) × available carbohydrates (total carbohydrates of the food [g] – fiber [g]). The value obtained was divided by the total content of ingested carbohydrates.

Participants were categorized into tertiles for the criteria and given a score (ranging from 1 to 3) for each tertile. For GI, those in the third tertile received 1 point, and those in the first tertile received 3 points. Finally, the CQI score was calculated by summing the values assigned to each category. The potential total score range varied from 4 to 12, with higher values indicating better carbohydrate quality, which were also categorized into tertiles. The categorization into tertiles instead of quintiles and scores from 1 to 3 were modifications of the initial methodology of the index due to the small sample size in this study (Table 2). However, sample division into tertiles has already been adopted in other studies [19].

## Statistical Analysis

Data were recorded using Excel® version 2010 software and later reviewed to assess consistency. All statistical

analyses were performed using STATA® software (STATA Corp, College Station, Texas, USA) version 13.0.

Differences between CQI tertiles were analyzed as follows. Qualitative variables were described as absolute and relative frequencies (percentage) and compared using the chi-squared ( $\chi^2$ ) or Fisher's exact test. The Shapiro–Wilk test was used for all continuous variables to analyze their normality and to determine the appropriate statistical test. Normally distributed quantitative variables are presented as means and standard deviations (SD), and groups were compared using parametric tests. Analysis of variance (ANOVA) for one factor followed by Tukey's post hoc was used to measure the differences between tertiles of the CQI. Quantitative variables without normal distribution are presented as medians and interquartile ranges (IQR) and compared using nonparametric tests the Kruskal–Wallis test followed by Dunn's post hoc test.

To compare the differences in the CQI and its components of the entire sample over the follow-up period (baseline and 3 and 12 months after surgery), a one-factor analysis of variance for repeated measures was used, followed by Bonferroni correction. Bivariate linear regression was performed to assess whether the CQI could predict BMI and insulin values 1 year after BS. For the regression model that included BMI and body fat (variables collected at 12 months), the CQI was added to the age and preoperative BMI, defined as an a priori adjustment based on the literature [20]. The BMI at 12 months was used as an adjustment for the regression analysis of insulin and HOMA-IR, considering its influence on insulin resistance. BMI and insulin level were considered dependent variables, and the CQI was independent.

## Results

From the total study sample, 58 participants were followed up for 3 months, and eight participants dropped out of the study for personal reasons. The remaining 50 patients were

**Table 1** Criteria used to calculate the carbohydrate quality index

CQI components	Index range (scores)	Criteria for minimum index	Criteria for maximum index
Dietary fiber intake (g/d)	1–3	Minimum fiber intake (1st tertile)	Maximum dietary fiber intake (3rd tertile)
Glycemic Index (GI)	1–3	Maximum glycemic index (1st tertile)	Minimum glycemic index (3rd tertile)
Ratio of whole grain/total grain carbohydrate	1–3	Minimum value of this relationship (1st tertile)	Maximum value of this relationship (3rd tertile)
Ratio solid carbs/(solid carbs + liquid carbs)	1–3	Minimum value of this relationship (1st tertile)	Maximum value of this relationship (3rd tertile)
Total index (range)	4–12	-	-

Source: Adapted from Zazpe et al. (2016) [16]

**Table 2** Sociodemographic, anthropometric, clinical, and food consumption characteristics of the participants according to the tertiles of the baseline carbohydrate quality index

Variables	Tertile 1 CQI	Tertile 2 CQI	Tertile 3 CQI	<i>P</i> value
<i>N</i>	18	24	8	-
<i>Baseline variables</i>				
CQI score*	<b>6.00 (5.00–7.00)<sup>a</sup></b>	<b>8.00 (8.00–9.00)<sup>b</sup></b>	<b>10.00 (10.00–10.05)<sup>c</sup></b>	<b>&lt;0.001</b>
Whole grains, % VET <sup>†</sup>	1.74 (3.24)	2.51 (4.23)	2.38 (2.63)	0.7924
Refined cereals, % VET <sup>†</sup>	16.91 (6.08)	17.52 (9.56)	13.06 (5.64)	0.3859
Solid carbohydrates, % VET <sup>†</sup>	<b>33.59 (7.71)<sup>a</sup></b>	<b>42.07 (10.11)<sup>b</sup></b>	42.82 (9.08) <sup>a,b</sup>	<b>0.013</b>
Liquid carbohydrates, % VET <sup>†</sup>	<b>11.26 (4.98)<sup>a</sup></b>	<b>5.55 (4.53)<sup>b</sup></b>	<b>3.04 (2.76)<sup>c</sup></b>	<b>&lt;0.001</b>
Glycemic index <sup>†</sup>	73.65 (23.83)	68.21 (31.50)	51.90 (9.99)	0.1658
Fiber, grams	<b>14.47 (4.96)<sup>a</sup></b>	19.72 (9.21) <sup>a,b</sup>	<b>26.97 (5.88)<sup>b</sup></b>	<b>0.001</b>
Age, years <sup>†</sup>	38 (7.66)	39.08 (7.83)	41.88 (8.99)	0.5225
Income, R\$*	5.750 (2.500–7000)	4.400 (2.050–8.500)	20.500 (2.850–46.000)	0.1759
Education, years <sup>†</sup>	16.69 (3.00)	15.73 (3.59)	16.19 (4.58)	0.6870
Sex female <i>n</i> (%) ‡	14 (77.78)	15 (62.50)	6 (75.00)	0.533
Civil status married/married alike <i>n</i> (%)‡	10 (55.56)	16 (66.67)	4 (50.00)	0.629
BMI, kg/m <sup>2†</sup>	42.04 (5.81)	42.53 (4.65)	42.06 (4.11)	0.9435
<i>Variables at 12 months of surgery</i>				
Weight, kg <sup>†</sup>	73.42 (10.52)	79.95 (12.71)	76.33 (10.31)	0.2042
Stature, meters <sup>†</sup>	1.63 (0.07)	1.67 (0.08)	1.66 (0.08)	0.2943
Weight loss, kg <sup>†</sup>	37.67 (8.29)	38.98 (12.25)	38.91 (9.97)	0.9182
BMI, kg/m <sup>2†</sup>	27.81 (4.60)	28.66 (3.60)	27.81 (4.60)	0.7474
Waist perimeter, cm <sup>†</sup>	88.81 (9.89)	96.33 (12.02)	89.44 (9.71)	0.0708
Neck perimeter, cm <sup>†</sup>	35.00 (4.18)	35.68 (3.18)	34.38 (3.32)	0.6391
Body fat, % <sup>†</sup>	29.16 (7.70)	29.23 (7.48)	30.21 (7.51)	0.9415
Fat-free mass, kg*	51.15 (47.10–53.90)	56.20 (47.40–64.10)	52.10 (49.25–59.55)	0.3324
Obesity remission 12 months, <i>n</i> (%)‡	12 (66.67)	16 (66.67)	6 (75.00)	0.898
Blood glucose, mg/dL <sup>†</sup>	83.45 (5.41)	83.29 (6.50)	84.21 (10.51)	0.9476
Total cholesterol, mg/dL <sup>†</sup>	153.63 (33.72)	153.64 (23.94)	160.65 (17.94)	0.8006
LDL cholesterol, mg/dL <sup>†</sup>	88.59 (31.71)	83.48 (22.22)	86.51 (15.01)	0.8076
HDL cholesterol, mg/dL <sup>†</sup>	52.07 (8.66)	53.15 (13.45)	59.76 (13.37)	0.3031
Ratio TC/HDL, mg/dL <sup>†</sup>	3.01 (0.78)	3.02 (0.73)	2.77 (0.52)	0.6756
Triglycerides, mg/dL <sup>†</sup>	67.50 (50–80)	85 (56.50–98.50)	65 (51.50–81.00)	0.1036
Ratio TG/HDL, mg/dL*	1.24 (1.03–1.42)	1.52 (1.14–2.29)	1.20 (0.94–1.49)	0.1158
Insulin, $\mu$ IU/mL <sup>†</sup>	<b>4.60 (2.58)<sup>a</sup></b>	3.96 (1.84) <sup>a,b</sup>	<b>2.37 (1.08)<sup>b</sup></b>	<b>0.037</b>
HOMA-IR <sup>†</sup>	0.94 (0.55)	0.82 (0.41)	0.50 (0.25)	0.0741
Insulin resistance, <i>n</i> (%)‡	1 (5.56)	0 (0.00)	0 (0.00)	0.404
Metabolic syndrome, <i>n</i> (%)‡	0 (0.00)	1 (4.17)	0 (0.00)	0.575

<sup>a</sup>Different letters mean statistically significant differences between groups. Bold  $P < 0.05$ . <sup>†</sup>Values in mean and standard deviation (SD) compared with one-way ANOVA followed by Tukey's post hoc; \*values in median and interquartile range (IQR) compared with Kruskal–Wallis test followed by Dunn's post hoc; ‡absolute and relative percentage values (%) compared with chi-square ( $\chi^2$ ) or Fisher's exact test. Abbreviations: CQI, carbohydrate quality index; R\$, Reais; kg, kilos; kg/m<sup>2</sup>, kilos per square meter; cm, centimeters; mg/dL, milligrams per deciliter; LDL, low-density lipoprotein; HDL, high-density lipoprotein; CT, total cholesterol; TG, triglycerides;  $\mu$ IU/mL, milli-international units per milliliter; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance. A total of 58 participants were followed up for 3 months, and eight participants dropped out of the study. The remaining 50 patients were followed up for 12 months.

followed up for 12 months. The study population consisted mainly of women aged between 38 and 42 years (Table 2). In the preoperative period, the mean BMI was 42 kg/m<sup>2</sup>, and excess weight ranged from 45 to 49 kg

Based on pre-surgical food consumption, the three groups were defined according to the CQI score, which differed significantly between the three groups (6 vs. 8 vs. 10;  $P < 0.001$ ). As for the components of the index, there were significant

differences in the consumption of solid carbohydrates, which was lower in tertile 1 than in tertile 2, and in fiber consumption, which was higher in tertile 3 than in tertile 1 (14.47 vs. 26.97;  $P=0.001$ ) (Table 2). At 12-month post-surgery, we observed no difference between the CQI tertiles, except for insulin, which was lower in tertile 3 (2.37  $\mu\text{IU/mL}$ ; SD 1.08  $\mu\text{IU/mL}$ ) in relation to tertile 1 of CQI (4.60  $\mu\text{IU/mL}$ ; SD 2.58  $\mu\text{IU/mL}$ ) ( $P=0.037$ ). The foods that contributed most to the consumption of each index component are listed in Supplementary Table 1. There were no statistically significant differences between the CQI tertiles at baseline (Supplementary Table 2).

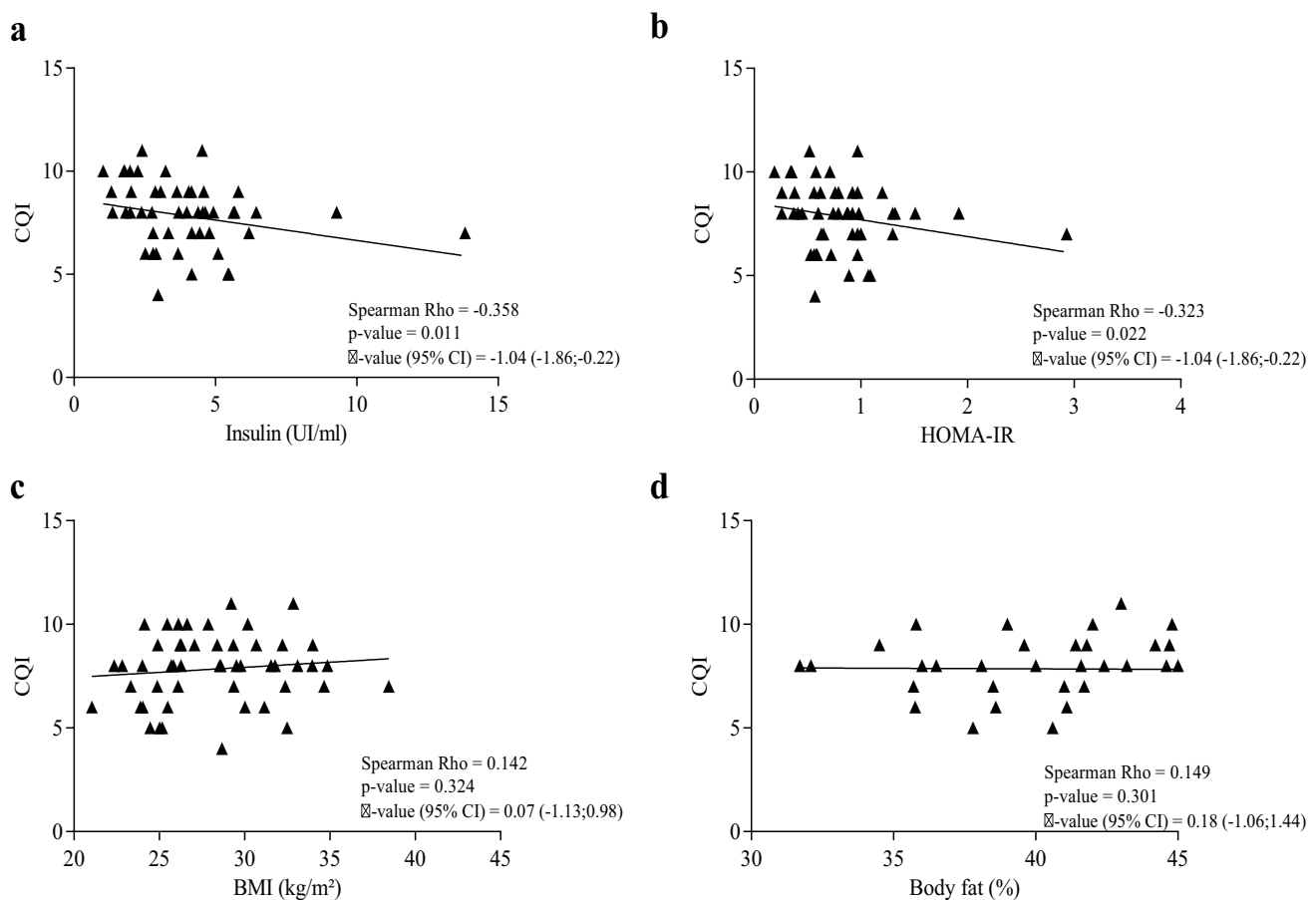
The results of linear regression analyses corroborated the comparison findings between the groups. A 1-unit increase in CQI was associated with a  $-1.02$  decrease in insulin concentration [CI  $-1.86$  to  $-0.17$ ,  $P=0.019$ ]. In the adjusted model, the reduction was even greater; 1-point increase in CQI promoted a  $-1.04$  decrease in insulin levels, regardless of BMI, at 12 months (Fig. 1). In the total sample, the median CQI was 8 points and did not change at 3 and 12 months after surgery.

The glycemic index increased in relation to 3 months. Refined carbohydrates liquids and solids decreased in relation to baseline, whose changes were maintained at 12 months (Fig. 2).

## Discussion

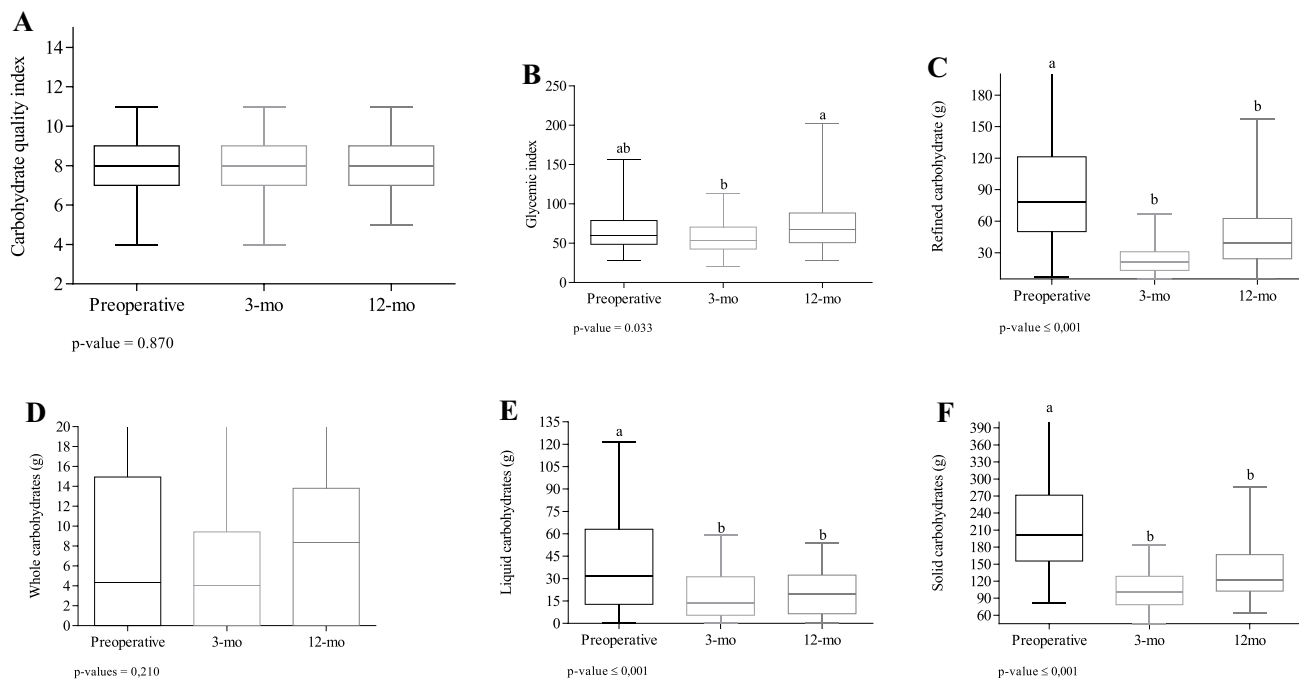
To the best of our knowledge, this is the first study to evaluate the quality of carbohydrates consumed using the CQI in patients undergoing BS. Our data suggest that carbohydrate quality (increased CQI) is associated with reduced serum insulin concentrations and the insulin resistance marker HOMA-IR. However, there was no relationship between BMI before and after the surgery. Furthermore, the data also indicate that carbohydrate quality did not change at 3 and 12 months after the surgical intervention, although there were positive changes in some of the CQI components.

Glucose is the main diet component that regulates insulin release and interferes with the function of pancreatic  $\beta$  cells



**Fig. 1** Associations between baseline carbohydrate quality index (CQI) with anthropometric, body composition, and clinical variables 12 months after RYGB. Rho and  $P$  values using Spearman correlation.  $\beta$  values and 95% confidence interval (CI) were obtained by multiple linear regression. Figures **a** and **b**  $\beta$  values adjusted by BMI

12 months after surgery; Figures **c** and **d**  $\beta$  values adjusted for age and pre-surgery BMI; 58 participants were followed up for 3 months, and eight participants dropped out of the study. The remaining 50 patients were followed up for 12 months



**Fig. 2** Difference between the carbohydrate quality index (CQI) and its components during the study's follow-up period (pre-surgery, 3 and 12 months after surgery). Note: P-values were obtained through repeated measures ANOVA. Different letters and equal letters mean statistically significant differences and the absence of statistically sig-

nificant differences, respectively, between groups, detected by Bonferroni's post-hoc correction. 58 participants were followed up for 3 months, and eight participants dropped out of the study. The remaining 50 patients were followed up for 12 months

[21]. However, there is no consistent evidence regarding the effect of carbohydrate restriction on insulin resistance [22]. This is because carbohydrates are a heterogeneous group that includes simple and complex carbohydrates, whole and refined carbohydrates, and fiber. Their physiological effects differ according to their quantity and quality [23]. This indicates the relevance of evaluation and guidance regarding the quality of carbohydrates in treating IR [22]. Consistent with this, epidemiological studies indicate that a diet rich in low glycemic load and whole grains improves insulin sensitivity and reduces the risk of developing type 2 diabetes mellitus (T2DM) [24]. In addition, higher CQI may help prevent metabolic syndrome in people with T2DM [19].

Dietary fiber has an important role to play in this by delaying gastric emptying and reducing the GI of food and, consequently, the postprandial glycemic response, as well as insulinemic “peaks” and the absorption of glucose and protein. Moreover, fibers also increase the production of short-chain fatty acids, promoting a positive modulation of the intestinal microbiota without contributing to caloric intake [25]. Together, these factors contribute to an increase in insulin sensitivity. Furthermore, other emerging mechanisms that justify these effects have been proposed, such as the modulation of intestinal hormones and regulation of adipokines and other markers of inflammation [26]

It is important to emphasize that there was no increase in the CQI (of the total sample) after surgery, despite some positive changes in some of the index components. The glycemic index even increased at 12 months, which may be due to the increase in the amount and variety of foods consumed, consistent with this, another study in post-bariatric patients indicated that the glycemic load, is directly correlated with caloric consumption [27]. This may indicate that the overall quality of the diet did not improve after BS because healthy diets tend to have better-quality carbohydrates, which favors a higher intake of micronutrients [17]. Furthermore, this result reinforces the need for long-term follow-up with nutritional counseling for these patients, as other studies have shown that diet quality worsens after BS [28].

Most studies evaluating macronutrient intake before and after BS have focused on protein intake, and studies evaluating carbohydrates quality are scarce. Considering that a portion of patients who undergo BS seek treatment due to the need to avoid complications of T2DM, the results are relevant because the factors that promote remission of IR after BS are not fully understood [29]. Therefore, it is important to guide the quality of carbohydrates, not only protein intake.

We also hypothesized that there may have been no association between BMI and CQI because, up to 12 months after surgery, many other physiological factors contribute to weight loss. However, evaluation at 24 months after surgery

would be relevant because the patient is more susceptible to environmental agents that generate weight regain in a more extended period of adaptation to post-surgical changes

This study has some limitations. First, information on food intake was from self-reports, which may generate measurement errors inherent to the instrument used. Still, careful application and validated tools were used to reduce this risk. Regarding strengths, this was the first study to evaluate the CQI in this population. Because of its design, it allowed us to discern temporal relationships between exposure and outcome.

## Conclusion

The data from the present study suggests that carbohydrate quality, as measured using the CQI, may contribute to the restoration of insulin sensitivity 12 months after RYGB. According to these findings, it is of utmost importance to guide patients to adopt a diet with whole complex carbohydrates and source of soluble and insoluble fiber, since many patients consume sugary drinks and other foods, which may negatively impact IR. Furthermore, considering that the focus in clinical practice is usually only on proteins, patients are not oriented to the quality of carbohydrates.

**Abbreviations** *BS*: Bariatric surgery; *CQI*: Carbohydrate quality index; *HOMA-IR*: Homeostatic Model Assessment of Insulin Resistance; *RYGB*: Roux-en-Y gastric bypass; *BMI*: Body mass index; *EBW*: Excess body weight; *WC*: Waist circumference; *NC*: Neck circumference; *BF*: Body fat; *GI*: Glycemic index; *R24*: 24-h recall; *SD*: Standard deviations; *IQR*: Interquartile ranges; *T2DM*: Type 2 diabetes mellitus; *IR*: Insulin resistance; *WHO*: World Health Organization; *FICT*: Free and informed consent term

**Supplementary Information** The online version contains supplementary material available at <https://doi.org/10.1007/s11695-023-06771-4>.

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**Author Contribution** Darlene Larissa de Souza Vilela contributed to the study design, formal analysis and interpretation of the data, drafting of the manuscript, and approval of the final version. Alessandra da Silva contributed to the study design, formal analysis and interpretation of the data, critical review of the manuscript, and approval of the final version. Sônia Lopes Pinto contributed to data collection and interpretation, supervision, critical review of the manuscript, and approval of the final version. Josefina Bressan contributed to study design, supervision, acquisition of funding for English revision, interpretation of data, critical review of the manuscript, and approval of the final version.

## Declarations

**Ethical Approval** All procedures performed in studies involving human participants were in accordance with the ethical standards of the insti-

tutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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

**Conflict of Interest** The authors declare no competing interests.

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