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Research Article

Effects of Prebiotic and Synbiotic Supplementation on Glycemic and Lipid Homeostasis after Roux-en-Y Gastric Bypass: A Randomized, Triple-Blind, Placebo-Controlled, Human Clinical Trial

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Abstract

Background

The supplementation with prebiotics and synbiotics has brought positive results in subjects with overweight and obesity, improving lipid and glycemic metabolic parameters.

Objectives

The aim of this study was to evaluate the effect of the prebiotic and symbiotic supplementation on these parameters in patients undergoing Roux-en-Y gastric bypass (RYGB) in an early postoperative moment.

Methods

Randomized, controlled, triple-blind trial, in which patients undergoing RYGB (n = 13) were supplemented with six g/day of placebo, prebiotics (fructooligosaccharides - FOS) or synbiotic (FOS + three strains of *Lactobacillos* and one of *Bifidobacterium*) for 15 days. The intervention started 30 days after surgery. The outcomes analyzed were fasting blood glucose and insulin, HOMA2-IR index and lipid profile. This trial was registered in Clinical Trials Platform under the identification number NCT02158676.

Results

Fasting blood glucose, fasting insulin, HOMA2-IR index and lipid profile (plasma concentrations of total cholesterol, LDL-c, HDL-c and triglycerides) outcomes showed no difference between the groups after supplementation ($p > 0.05$).

Conclusion

The post-bariatric surgical condition appears as a new challenge taking into consideration the several factors that affect the gastrointestinal tract, to seek how to manage and provide benefits to patients regarding the optimal timing for supplementation, the duration of treatment, as well as the type and dose of the prebiotic and synbiotic to be used. This study shows no significant effects on metabolic parameters in individuals subjected to RYGB after 15 days of prebiotics and synbiotics supplementation initiated 30 days after surgery.

Keywords: Bariatric Surgery; Prebiotic; Symbiotic; Blood Glucose; Lipid Profile; Random Clinical Trial

Abbreviations: RYGB - Roux-en-Y Gastric Bypass; FOS – Fructooligosaccharides; HOMA2-IR- Homeostatic Model Assessment; LDL-c - Low-Density Lipoprotein Cholesterol; HDL-c - High-Density Lipoprotein Cholesterol; DM2- Type 2 Diabetes Mellitus; BMI - Body Mass Index; TC- Total Cholesterol

Introduction

A global crisis of metabolic health has been caused by the obesity epidemic. For the first time in two centuries, there is a reduction in life expectancy associated with unhealthy body mass index (BMI) due to comorbidities that obesity causes [1]. The reduction in body weight has been managed with low solvability by traditional therapies. Bariatric surgeries have been shown to be important tools to combat obesity, promoting weight loss and improving healing and comorbidities [2-3].

The obesity negatively impacts the energy metabolism and inflammatory response. Disturbances in glucose homeostasis and lipid profile may occur as a result of weight gain, causing diseases such as type 2 diabetes mellitus (DM2), cardiovascular disease, dyslipidemia, liver disease, inflammatory bowel disease and certain cancers, thus damaging organs like pancreas, liver, gastrointestinal tract itself, among other organs and tissues [4].

Human gut microbiota, microorganisms which reside mainly

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in the lower gastrointestinal tract, performs several functions in mediating various metabolic pathways, as well as influencing the intestinal transit and the digestion of food. The imbalance of this microbiome, called dysbiosis, has been assigned to certain cases of disease and body weight of individuals [5]. Both obesity and bariatric surgery impact qualitatively and quantitatively in populations of bacteria of the gutmicrobiota [6]. Prebiotics and synbiotics, potential modulators of the human gut microbiota have been studied for reverting dysbiosis states, favoring the reestablishment of gut bacteria in the host, thereby generating metabolic benefits [7]. Assays performed in obese subjects have shown significant results in recovering metabolic homeostasis pathways [8].

This study investigates the supplementation of prebiotics and synbiotics in patients undergoing a type of bariatric surgery, the Roux-en-y gastric bypass. The supplementation started 30 days after surgery, remaining for 15 days, allowing evaluating the metabolic changes of both the effect of the surgical procedure and of the intervention on the studied outcomes, seeking a better understanding of the influence of modulators of gut microbiota in these moments.

Materials and Methods

Design and Study Participants

Randomized clinical trial, placebo-controlled, triple blind, conducted from October 2013 to November 2014 at the University Hospital Professor PolydoroErnani de São Thiago, Federal University of Santa Catarina, Florianópolis, in which were evaluated 13 individuals undergoing RYGB. Inclusion criteria were: individuals of both sexes aged between 19 and 60 years, BMI> 40 kg/m^2 or> 35kg/m^2 with at least one comorbidity, failure in clinical treatment of obesity and to undergo bariatric surgery by open Roux-en-Y technique. Exclusion criteria were: significant intellectual limitations without proper family support; uncontrolled psychiatric disorder; alcohol dependence and/or illicit drugs; smoking; use of anti-inflammatory drugs and/or antibiotics in the last 3 months; intolerance to prebiotic and/or probiotic and/or synbiotic; use of a prebiotic and/or probiotic and/or synbiotic in the last 3 months.

All participants received the same interdisciplinary education about the risks and changes in habits inherent to a major surgery in the digestive tract and the need for postoperative changes in lifestyle. All patients also received the same nutritional guidelines with changing diets after surgery.

This study was approved by the Ethics Committee on Human Research of the Federal University of Santa Catarina, which complies with the World Medical Statement of Helsinki [9], under the protocol number 245 650/2013. Participants were invited to participate and those who accepted signed an Informed Consent. This trial was registered in Clinical Trials Platform under the identification number NCT02158676.

Moments and Intervention Groups

The clinical trial had two experimental moments to individuals involved: M1 - 30 days after surgery and the start of supplementation with prebiotic, synbiotic or placebo; and M2 - after 45 days of surgery and fifteen days after the start of the supplementation.

Participants were asked to consume six grams of placebo (maltodextrin), six grams of prebiotic (fructo-oligosaccharide (FOS) - FiberFOS®, InvictusFarmanutrição, Brazil) or six grams of synbiotic (FOS, 1x10^9*Lactobacillus paracasei* LPC-37, 1x10^9*Lactobacillus rhamnosus* HN001, *Lactobacillus acidophilus* NCFM 1x10^9 and 1x10^9*Bifidobacteriumlactis* HN019 - LactoFOS®, InvictusFarmanutrição, Brazil) daily for 15 days, mixing the contents at 100ml of water until complete dilution and consume in fasting state. The control of the intake was performed by registration in a specific form provided by the researchers. Researchers randomly assigned the participants to treatment groups using a randomization list generated by program computer consisting of randomly permuted blocks with three individuals each. All individuals evaluated were assigned to the treatment arm according to the randomization number. A copy of the randomization sequence was kept in a locked cabinet apart from the study personnel. Study participants and researchers were blinded with respect to the consumption and distribution of supplementation, respectively. The supplements and placebo were pre-packed by the supplier with randomization codes, being identical in physical appearance, flavor and color. The identification codes of the supplements were revealed by the manufacturer only after data analysis

Outcomes

The primary outcomes were evaluation of fasting insulin and glucose, HOMA2-IR index and plasma lipid profile of individuals after supplementation. The lipid profile was analyzed by the plasma concentrations of total cholesterol, HDL-cholesterol, LDL-cholesterol and triglycerides. Surgery outcomes were assessments of weight and body mass index after surgery and after supplementation and the evaluation of fasting insulin and glucose, HOMA2-IR index and lipid profile of individuals after surgery until the time of supplementation. Fasting blood glucose was determined by enzymatic method, according to the manufacturer's instructions (Dimension RxL Max® - Siemens Healthcare Diagnostics Inc. Newark, DE, USA. Insulin was determined by immunometric assay chemiluminescent enzyme labeled solid phase using the Immulite kit Systems® 2000 (Siemens Healthcare Diagnostics Inc., Newark, DE, USA). The HOMA2-IR (Homeostasis model assessment 2 – insulin resistance) index was calculated with fasting blood glucose and insulin, using HOMA2 Calculator program v.2.2.3 © (Diabetes Trials Unit, University of Oxford).

Total cholesterol, HDL-c and triglycerides were measured by enzymatic colorimetric method, AHDL and TGL respectively, according to the manufacturer's instructions (Dimension RxL Max® - Siemens Healthcare Diagnostics Inc. Newark, DE, USA). The LDL-cholesterol was estimated using the Friedewald equation

(LDL-c = total cholesterol - HDL-c - Triglycerides / 5) [10].

Data and Statistical Analysis

Statistical analysis was performed using statistical software Stata® (StataCorp, Texas, USA), version 11.0. Raw data is displayed in mean and standard deviation. Comparisons between groups were performed using analysis of variance (ANOVA) and the Kruskal Wallis test according to the normality of data. For intra group comparison, the paired T test or Wilcoxon test were used.

Results

33 patients underwent RYGB were evaluated for eligibility from October 2013 to April 2014 and between August and November 2014. Ten patients were excluded due to antibiotic use (7), refused to participate (2) and smoke habit (1). Finally, 23 patients were randomized to the intervention and 13 patients finished the clinical trial. There were 10 losses during the clinical trial due to antibiotic use (3), errors in sample collection (2) and 4 patients gave up from the study. The selection, losses and patients included in the study are shown in Figure 1.

Characteristics of the Study Participants

Baseline characteristics of the participants, for the pre-surgical moment are shown in Table 1. No adverse effects were reported related to maltodextrin consumption in the placebo group, while one individual reported increase of gas during the early days of supplementation; however it did not result in the withdrawal of the study.

Participants reported that they followed the prescribed diet during the supplementation period (800 kcal/day, with balanced reduction of all macronutrients and no consume of alcoholic beverages, carbonated drinks or sugar) and did not reported complications to dietary intake. After surgery, a protocol of medications and supplements was used for all the participants, including omeprazole administration (40 mg orally per day) vitamin and mineral supplement and injectable supplementation of vitamin B12 (5000 µg). The use of all medications for comorbidities shown in Table 1 were removed after RYGP, with the exception of administration a hypolipidemicin one subject in group one.

Fasting Glucose, Fasting Insulin and HOMA2-IR

Fasting blood glucose, fasting insulin and HOMA2-IR showed no difference in analysis between the groups and neither intra group (Table 2). The means of means differences of plasma glucose concentration were -2.4 mg / dL (95% CI -9.1, 4.3) in the placebo group, 3.5 mg / dL (95% CI -8.8, 15.8) in the prebiotic group and -6.5 mg / dLL(95% CI: -30.4, 17.4) in the synbiotic group. The mean of means differences (95% CI) inplasma insulin concentration were -1.6 µIU / mL (95% CI -12.5, 9.2) in the placebo group, 2.5 µIU / mL (95% CI -10.1, 5.1) in the prebiotic group and -2.2 µIU / mL (95% CI -6.7, 2.3) in the symbiotic group. The absolute values of fasting blood glucose and insulin, and the means of means differences showed great dispersion between groups, not pointing any trend or pattern of results (Figure 2).

Lipid Profile

The plasma total cholesterol (TC), LDL-c, HDL-c and triglyceride concentrations had no significant difference between groups. However, in the intra group analysis, there was a significant reduction in total cholesterol in the group supplemented with placebo ($p = 0.005$) as well as a trend towards reduction in LDL-c ($p = 0.100$) and triglyceride ($p = 0, 065$) in the same group. The mean of means difference of TC were -26.6 mg / dL (95% CI -40.0; -13.3) in the placebo group, 3, 3 mg / dL (95% CI -30 8; 24.3) in the prebiotic group and 6.3 mg / dL (95% CI: -13.0, 25.5) in symbiotic group. The mean of means difference of LDL-c were -16.0 mg / dL (95% CI: -25.7, -6.3) in the placebo group, -10.0 mg / dL (95% CI -41.8, 21.8) in the prebiotic group and 4.5 mg / dL (95% CI: -10.6, 19.6) in symbiotic group. The mean of means difference of triglycerides were -23.4 mg / dL (95% CI: -49.2, 2.4) in the placebo group, 8.25 mg / dL (95% CI: -55.1; 72.2) in the prebiotic group and -3.0 mg / dL (95% CI -27.3, 21.3) in the symbiotic group.

In the groups supplemented with prebiotic and symbiotic there was no significant difference in the parameters of the lipid profile in the intra-group analysis. In the group supplemented with prebiotic three subjects had reductions in absolute values in TC concentrations, LDL-c and triglycerides, but other individual in the group had an increase in these three parameters (Figure 2).

Surgery Outcomes

All metabolic parameters showed no significant difference between groups in the pre-surgical time. Between the surgery time and the start of supplementation, there was a significant reduction in total cholesterol ($p = 0.008$), LDL cholesterol ($p = 0.009$) and triglycerides ($p = 0.014$) in the placebo group (paired T test). The intra group analyzes in the groups supplemented with synbiotic and prebiotic showed no significant differences. Body weight and BMI showed no significant differences between groups in any moments.

Discussion

The results of this trial indicate that the supplementation points to a lack of effect on metabolic parameters of individuals. Major changes in lipid and glucose homeostasis that occur after surgery have not changed with the supplementation of prebiotic and synbiotic by the study design adopted in this clinical trial. The absence of effect can also be attributed to the particular characteristics of the pre-bariatric surgical patient, the type of supplement used, as well as the treatment time and dose.

The RYGB, surgical technique performed in all patients in the study, implies in profound anatomical and physiological changes in the gastrointestinal tract [6]. These changes, along with the important change to the food intake after surgery, cause changes in the resident microbiota due to this distinct ecological niche created in the intestines and stomach [12].

In relation to the upper part of the gastrointestinal tract, the surgical procedure creates a blind loop including parts derived from stomach and the biliopancreatic limb, creating a potential source of bacterial overgrowth in the area of stasis (no food flow) and also in

Characteristics	Placebo (n=5)	Prebiotic (n=4)	Symbiotic (n=4)	P
Age (years)	40.6 ± 12.0	42.5 ± 6.9	41.2 ± 13.5	0.968
Sex (Male/Female)	0/5	1/3	0/4	
Body weight (kg)	112.6 ± 4.5	127.3 ± 30.8	122.0 ± 20.3	0.567
Body mass index (kg/m²)	43.1 ± 3.4	46.6 ± 7.4	44.2 ± 6.3	0.662
Medications (n)				
Antihypertensives/diuretics	4	3	3	NA
Oral hypoglycemics	1	2	1	NA
Hypolipemiants	1	1	1	NA
Comorbidities (n)				
Hypertension	4	3	3	NA
Diabetes mellitus type 2	1	2	1	NA
Dyslipidemia	1	1	1	NA
Hepatic steatosis	1	2	1	NA
Gastritis	2	0	1	NA
Metabolic parameters				
Glucose (mg/dL)	99.8	98.0	100.5	0.991
Insulin(µIU/mL)	18.0	19.0	13.8	0.410
HOMA2-IR	2.3	2.4	1.8	0.563
TC (mg/dL)	192.6	183.0	147.5	0.403
LDL-c (mg/dL)	145.0	116.3	107.3	0.453
HDL-c (mg/dL)	37.8	46.0	34.3	0.255
Triglycerides (mg/dL)	150.8	158.5	117.5	0.527

Table 1: Baseline characteristics of individuals (pre-surgical moment).

Metabolic parameters (fasting)	Placebo(n=5)		Prebiotic (n=4)		Symbiotic (n=4)	
	Before treatment	After treatment	Before treatment	After treatment	Before treatment	After treatment
Glucose (mg/dL)	98.6 ± 2.7	96.2 ± 6.9	97.8 ± 19.0	101.3 ± 24.8	90.5 ± 11.7	84.0 ± 8.1
Insulin (µIU/mL)	13.6 ± 3.2	12.5 ± 3.4	19.5 ± 11.9	17.0 ± 8.8	14.3 ± 6.3	12.1 ± 5.0
HOMA2-IR	1.6 ± 0.3	1.6 ± 0.5	2.5 ± 1.5	2.2 ± 1.2	1.8 ± 0.8	1.5 ± 0.6
TC (mg/dL)	171.2 ± 32.9	144.6 ± 26.3	155.5 ± 25.0	152.3 ± 20.7	133.3 ± 35.7	139.5 ± 39.6
LDL-c (mg/dL)	113.6 ± 28.9	97.6 ± 22.6	98.0 ± 28.6	88.0 ± 24.1	86.8 ± 30.6	91.3 ± 35.5
HDL-c (mg/dL)	33.8 ± 4.0	33.6 ± 4.6	38.5 ± 7.3	38.5 ± 6.4	30.0 ± 6.1	31.3 ± 6.2
Triglycerides (mg/dL)	112.4 ± 38.9	89.0 ± 27.7	114.3 ± 38.2	122.5 ± 77.7	117.3 ± 74.4	114.3 ± 63.8

Table 2: Metabolic parameters of subjects before (30 days after surgery) and after (45 days after surgery) supplementation.

nearest parts the other limbs [12,13]. There still two other factors that influences these niches and that are linked to bacterial shifts, specially the increase in the phylum *Proteobacteria* [14-16].The first is the change in the flow of bile acids, with possible increase of its concentration regarding the lower amount of bolus in the lower gastrointestinal tract, which may create a more favorable niche to bacterial groups more tolerant to bile [17]. The second factor is the changes in oxygen supply between segments of the gastrointestinal tract related to the derivations and sections of the surgical process. The distal jejunum, closer to the beginning of the gut can change the availability of oxygen transforming bacterial niche of the intestine, possibly favoring facultative anaerobic bacteria, most common in the phylum *Proteobacteria*, even more predominant in class *Gammaproteobacteria*class [18]. This could be also a disadvantage to bacteria of *Bifidobacterium* genre due to its anaerobic restrict characeteristic [19]. Those changes in the gastrointestinal tract may alter and/or prevent the action of the present study intervention, suggesting that the potential beneficial effects of prebiotic and synbiotic might depend on a more preserved gastrointestinal anatomy.

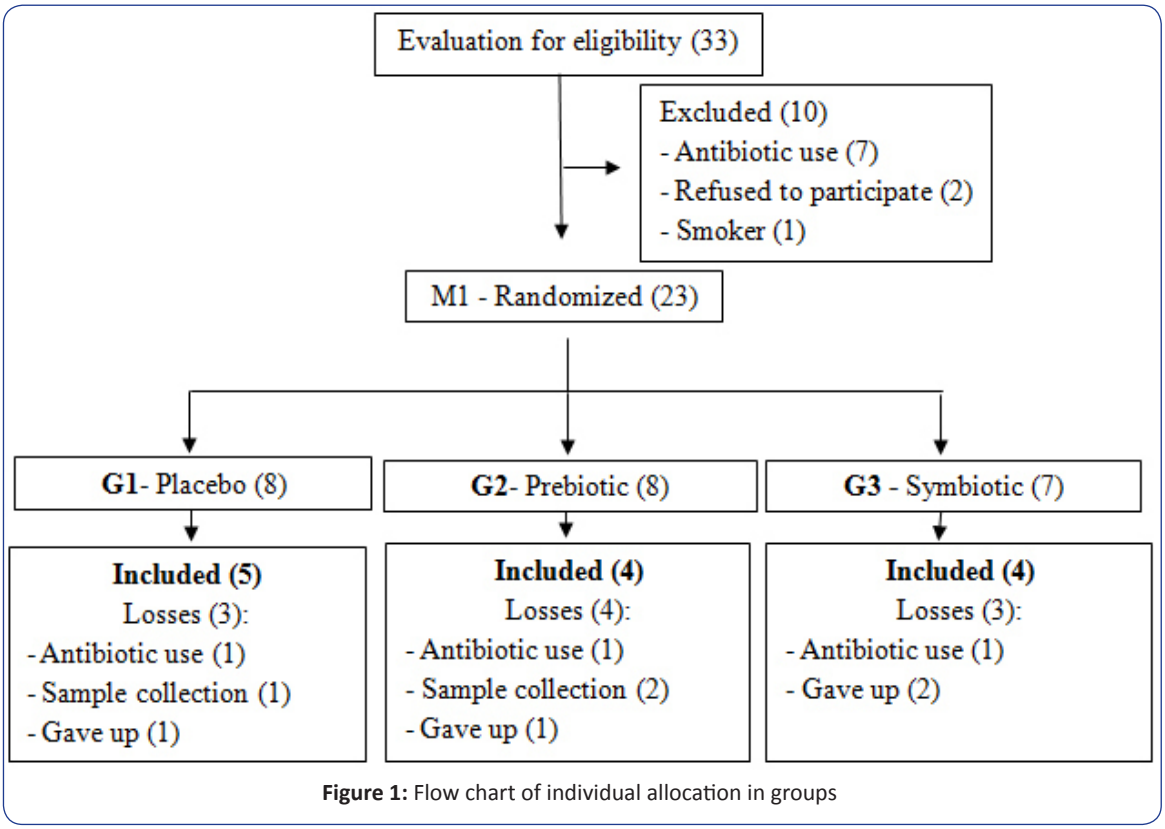

Figure 1: Flow chart of individual allocation in groups

The synbiotic used in this trial contained four bacterial strains, *Lactobacillus paracasei* LPC-37, *Lactobacillus rhamnosus* HN001, *Lactobacillus acidophilus* NCFM and *Bifidobacteriumlactis* HN019, and beneficial effects of these probiotics have been identified to species and strain levels, especially in the lipid profile in hypercholesterolemic individuals with overweight and obesity [20-24]. In contrast, the present study showed no changes in lipid and glucose profile of the participants with supplementation of synbiotic with such species and strains.

In addition to the aforementioned changes in oxygen supply and increased phylum *Proteobacteria*, which causes direct competition for colonization sites, the change in intestinal pH may be disadvantaged specifically to *Lactobacillus* and *Bifidobacterium* genera due to their acid uric characteristic of better proliferation between pH of 5.5 to 6.0 and 6.0 to 7.0, respectively [19]. The RYGB promotes a decrease in gastric acid secretion [25], which combined with the protocol use proton pump inhibitors after surgery in all of the study patients, could have increased the pH [26] and prevented the colonization of strains used in this study and decreased the concentrations of these genres in gut microbiota as observed in other studies [11].

Another special characteristic of the study patients is its trajectory until surgery. Participants had their surgeries performed by the Unified Health System of Brazil, where multidisciplinary follow-up protocols of at least two years were applied. This included a nutritionist that guides the patient over these two years on a reduced calorie diet of 1200 kcal, which along drug therapies helps to control the metabolic profile of these individuals. This can be evidenced in the initial characteristics of the participants of which only two of a total of 13 subjects (15.4%) had total cholesterol> 240 mg/dL, two had LDL-C >160 mg/dL, two showed triglycerides>200 mg/dL and also only two subjects had fasting glucose above >126 mg/dL. This may have been another factor contributing to the lack of effect of the intervention on these parameters.

Regarding the duration and dose of the intervention, a direct comparison is not possible due to absence of similar studies. A meta-analysis pooled the results of trials that supplemented prebiotic and synbiotic in overweight and obesity, finding beneficial effects of prebiotic supplementation on total cholesterol and LDL-c in the general analyzes and HDL-c and triglycerides in a sub-analysis in diabetic subjects, still, symbiotic supplementation improved fasting insulin and the triglycerides concentrations [8]. The prebiotics doses of these studies varied between 1.08 and 21g, while the dose of probiotics contained in synbiotic was 10^8-10^9 UFC, however, analyzing the studies individually, there was not a linear response of the beneficial results obtained in relation to the dose. Regarding the treatment duration, six studies that showed beneficial effects adopted between 60 and 198 days of intervention, while the other seven studies ranged between 28 and 90 days, indicating that the longer supplementation can enhance maintenance of effects in overweight and obesity.

However, the dose of 6g of FOS in the prebiotic and synbiotic is closer to researches above mentioned than the treatment duration of 15 days on this study. Despite that the gut microbiota could be modulated quickly by diet [17] and supplements [7,27], these metabolic outcome analyzed may need a more robust intervention,

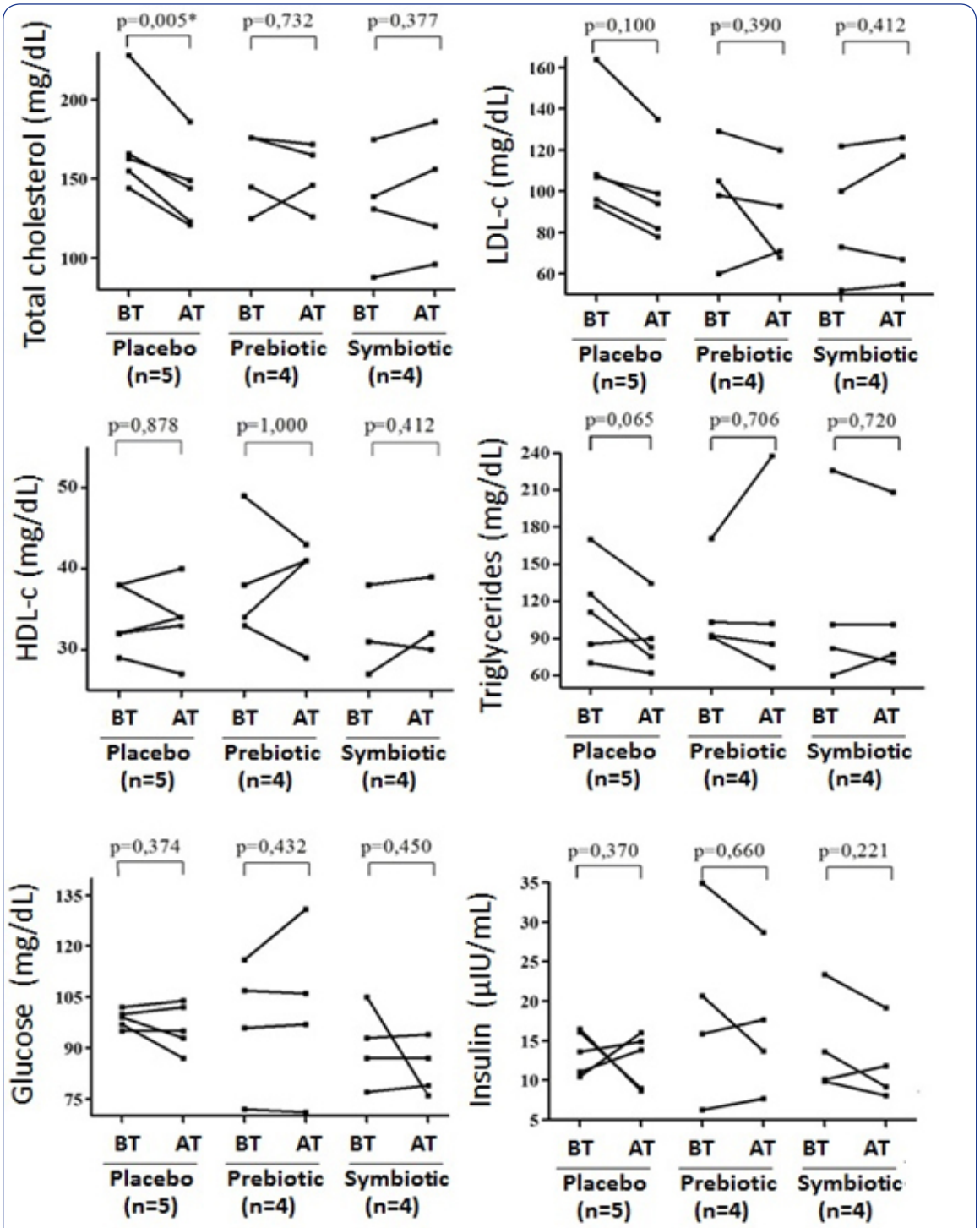

Figure 2: Metabolic parameters of each individual and intragroup analysis before (30 days after surgery) and after (45 days after surgery) supplementation

particularly concerning the treatment time, to obtain significant changes. The small sample size achieved in this trial is a factor that may have hindered the investigation of outcomes and also hampers the postulation of possible elements that led to lack of improvements in metabolic parameters.

During the supplementation period there was an intra group significant change on TC concentrations in the placebo group ($p < 0.05$) and a tendency to reduction in LDL-c ($p = 0.100$) and triglycerides ($p = 0.065$). These same outcomes were the ones that also showed a significant reduction between the pre-surgical time and the start of supplementation (p values of 0.008, 0.009 and 0.014 for total cholesterol, LDL-C and triglycerides, respectively) and this difference was observed only in the placebo group, highlighting

the absence of effects of the supplemented groups, suggesting the natural course of postoperative effects on improvement of these parameters.

Lack of control of food intake of participants is a limiting factor of this research, due to the fact that each participant can eat different daily values of energy and nutrients through diet. However, patients undergoing Roux-en-Y gastric bypass have specific dietary restrictions already discussed above, especially in the first postoperative stages, making the food intake more homogeneous among the groups.

Conclusion

Modulations of gut microbiota by supplementation of prebiotics, probiotics and synbiotics have increasingly shown the need for full knowledge of every situation to achieve positive therapeutic effects. This study shows no significant effects on metabolic parameters in individuals subjected to RYGB after a 15 days supplementation of prebiotics and synbiotics, started 30 days after surgery. Nevertheless, post-bariatric surgical condition is presented as a new challenge, considering the several factors that affect the gastrointestinal tract, to seek how to intervene to provide benefits to patients regarding the optimal duration of supplementation, the suitable moment for treatment, as well as the type and dose of microbiota modulator supplement to be used.

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