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Pilot study

Oral supplementation with L-glutamine alters gut microbiota of obese and overweight adults: A pilot study

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ABSTRACT

Objective: The aim of this study was to determine whether oral supplementation with L-glutamine (GLN) modifies the gut microbiota composition in overweight and obese adults.
Methods: Thirty-three overweight and obese adults, ages between 23 and 59 y and body mass index between 25.03 and 47.12 kg/m^2, were randomly assigned to receive either oral supplementation with 30 g of L-alanine (ALA group control) or 30 g of GLN (GLN group) daily for 14 d. We analyzed the gut microbiota composition with new-generation sequencing techniques and bioinformatics analysis.
Results: After 14 d of supplementation, adults in the GLN group exhibited statistically significant differences in the Firmicutes and Actinobacteria phyla compared with those in the ALA group. The ratio of Firmicutes to Bacteroidetes, a good biomarker for obesity, decreased in the GLN group from 0.85 to 0.57, whereas it increased from 0.91 to 1.12 in the ALA group. At the genus level, *Dialister*, *Dorea*, *Pseudobutyrivibrio*, and *Veillonella*, belonging to the Firmicutes phylum, had statistically significant reduction.
Conclusion: Oral supplementation with GLN, for a short time, altered the composition of the gut microbiota in overweight and obese humans reducing the Firmicutes to Bacteroidetes ratio, which resembled weight loss programs already seen in the literature.

Introduction

Obesity is a serious public health problem that affects millions of individuals worldwide [1]. This condition alters the diversity of the gut microbiota and consequently how individuals extract energy from nutrients and store these calories in adipose tissue [2]. It is well known that gut microbiota plays an important role in modulating digestive, endocrine, and immune systems [3]. For a healthy individual, the microorganisms that reside in the gut assist the capture of energy from food through the fermentation of nondigestible food components. Moreover, they provide protective effects on the intestinal epithelium and the immune system [4]. However, an imbalance of these microbial communities can lead to negative consequences including intestinal inflammation, allergies, infection, cancer, gastrointestinal disorders, and obesity [5,6].

Studies suggest that the transplantation of gut microbiota from obese to nonobese germ-free mice yields changes in energy expenditure, food intake, and absorption of energy from food [6,7], resulting in the transfer of metabolic syndrome–associated features from the donor to the recipient [5,8]. Over the past few years, researchers have extensively investigated the link between gut microbiota and obesity [6,9–12]. Although the details of this relationship are still unclear, recent research in this area demonstrates the existence of an interaction between

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microbiota and diet [11]. It is known that the amino acid L-glutamine (GLN) plays a physiological role in the gut and contributes to a nutritionally important portion of intestinal energy generation [13,14]. However, to our knowledge, there has been no thorough investigation of the effects of GLN on the gut microbiota. Consequently, interventional studies are needed to improve the understanding of how nutrients may interfere in the composition of the gut microbiota and hence, decipher how its manipulation can be targeted for nutritional and pharmacologic intervention in the treatment of obesity. Therefore, the aim of this study was to investigate whether oral supplementation with GLN alters the composition of the gut microbiota of overweight and obese adults. To the best of our knowledge, this is the first complete investigation about the glutamine effect on human gut microbiota.

Methods

All procedures were approved by the Ethics Committee of the School of Medical Sciences at the State University of Campinas, Campinas, São Paulo, Brazil, and were conducted in accordance with the Declaration of Helsinki (1964). All participants provided written consent before their enrollment.

Study design

The study was conducted at the State Hospital of Sumare, in Sumare city, São Paulo state, Brazil. All volunteers were employees in the hospital and were randomly recruited under the following criteria: age between 20 and 60 y and body mass index (BMI) ≥25.0 kg/m^2. The exclusion criteria included renal or thyroid disease; hormonal problems; pregnancy; use of antidepressants, laxatives, anorectic drugs, antibiotics or a combination of these agents within 2 mo before enrollment.

In this double-blind, 14-d study, participants were randomly divided into two groups: glutamine (GLN) and alanine (ALA). We used alanine as control to give the volunteers the same amount of calories. Participants received a kit containing small packs with 15 g of amino acid (GLN or ALA) each, with varying artificial flavors, to be diluted in 200 mL of water at the time of intake. Participants were instructed to take two packs at any convenient time of the day, totaling 30 g/d of amino acid, while maintaining their usual diets and physical activities.

Clinical measurements and biochemical analysis

Overnight-fasted volunteers came to the hospital in the morning on two separate days, baseline (day 0) and day 14, for blood sample collection and body measurements. Body weight and height were measured using a Filizola scale with an anthropometer (PL 200 model). BMI was calculated by dividing weight by height squared (kg/m^2) and waist circumference (WC) was measured in cm at a level midway between the lowest rib and the iliac crest.

A 24-h food record was documented before and after supplementation, together with the food diary of three consecutive days half way into the study. We used Dietpro Nutrition 4.0 software to compute the dietary data.

Blood samples from the volunteers were collected into tubes, which were then placed on ice and immediately centrifuged at 1500*g* for 15 min at 18°C using a Centrifuge BiofugeStratos (Hereaus, DijkstraVereenigde, Lelystad, Netherlands). Glucose concentration was determined by the glucose oxidase method.

Gut microbiota analysis (new-generation sequencing)

To assess gut microbiota, samples of ~5 g of feces were collected from all participants at baseline and after the 14-d treatment, using sterile stool containers and gloves. Feces were collected at any time of the day and taken to the laboratory for storage within 24 h of collection. All samples were stored in sterile tubes at −80°C until use. Total bacterial DNA was extracted from the fecal samples using the QIAamp DNA Stool Mini kit (Qiagen, GmbH, Germany) according to the manufacturer's protocol. DNA concentration and quality in the extracts were determined by agarose gel electrophoresis with a NanoDrop 1000 spectrophotometer (Thermo Scientific, Waltham, MA, USA).

Twenty-two primers were designed to sequence the V3 region of the *16S rRNA* gene, using the Forward 338 F and Reverse 533 R positions. The primers were used to amplify the DNA sequence using Platinum Taq High Fidelity polymerase chain reaction. The preparation of the genomic sample was made with Nextera XT kit, and the sequencing was run on IlluminaMiSeq platform. The experiment was designed to obtain overlapping fragments paired end 250 base pairs and generated an output of 5 Gb.

To determine which organism the sequence originated, we used rdp_classifier Software and database from the Ribosomal Database Project, adopting the percentage of similarity of 80%.

Statistical analysis

Statistical analyses were performed using the statistical software R (www.r-project.com) and SAS (SAS Institute Inc., Cary, NC, USA); the data were processed using Microsoft Excel. Continuous variables were expressed as the mean and SD. Comparisons for continuous variables between the groups were performed with the Student's *t* test for parameters with a normal distribution, which was tested with the Anderson-Darling normality test. If the normality was not satisfied, comparisons between groups were made with the Wilcoxon Mann–Whitney test. Additionally, we performed an advanced statistical procedure previously proposed [15]. We considered the counts of bacteria at each time point, baseline and day 14, as two random samples (X, Y) and specified the null hypothesis H_0 as follows: The mean count of bacteria is the same in both time periods. The counts were modeled by negative binomial (NB) distributions, BN (a_X, b) and BN (a_Y, b) respectively, to deal with over dispersion. In this setup, the null hypothesis can be written as H_0: $a_X = a_Y$. Then, the likelihood under the null L $(x, y|a_X = a_Y = a, b)$ and alternative L $(x, y|a_X, a_Y, b)$ hypotheses were computed and a generalized likelihood ratio test (GLRT) was performed for both groups separately, i.e.,

$$v_{GLN}(x,y) = \frac{\sup L(x,y|a_X = a_Y = a,b)}{\sup L(x,y|a_X,a_Y,b)}$$

$$v_{ALA}(x,y) = \frac{\sup L(x,y|a_X = a_Y = a,b)}{\sup L(x,y|a_X,a_Y,b)}$$

Because the approximate distribution of the likelihood ratio test $v(x, y)$ is χ^2 with 1 degree of freedom, the *P*-value was computed using the χ^2 95th percentile. $P < 0.05$ was considered significant.

Results

In all, 33 patients with a mean age of 38.5 (23–59) y and BMI between 25.03 and 47.12 kg/m^2 were enrolled in the study. Originally 74 women and 4 men initially volunteered for the study. At the end of the supplementation period, there were 21 participants in the GLN group and 12 in the ALA arm. In all we had 32 women and 1 man participating. Both ALA and GLN groups were comparable in many clinical and laboratory characteristics at baseline and at the end of study. There were no significant differences in body weight, BMI, WC, or fasting glucose between the groups (Table 1). Table 2 shows the average macronutrient and kcal intake computed using the 24-h food record before and after supplementation, together with the food diary of three consecutive days half way into the study. The results suggest that, kcal, lipid, protein, and carbohydrate consumption did not differ statistically between the groups (Table 2).

Table 1
Participants' metabolic characteristics

	ALA group (n = 12)		GLN group (n = 21)		*P*-value*
	Before	After	Before	After	
Height (m)	1.62 ± 0.07		1.59 ± 0.06		0.15
Weight (kg)	78.3 ± 16.0	78.4 ± 15.7	86.7 ± 15.2	86.7 ± 15.2	0.59
BMI (kg/m^2)	29.3 ± 4.4	29.4 ± 4.2	34.4 ± 5.9	34.4 ± 5.9	0.57
WC (cm)	90.2 ± 9.2	88 ± 7.8	96.9 ± 11.9	94.8 ± 11.6	0.84
Glucose (mg/dL)	84 ± 7.9	84 ± 8.9	79 ± 9.1	83 ± 10.5	0.11

ALA, alanine; BMI, body mass index; GLN, L-glutamine; WC, waist circumference
Data were collected before and after 14 d of supplementation with ALA or GLN. Serum glucose levels were obtained from fasted individuals. Data are expressed as mean ± SD

* *P*-value from unpaired Student's *t* test under normality and Wilcoxon Mann–Whitney test otherwise.