Oral Supplementation with L-Glutamine Alters Gut Microbiota of Obese and Overweight Human Adults: A Pilot Study

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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/m², 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
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 Biofuge Stratos (Hereaus, Dijkstra Vereenigde, Lelystad, Netherlands). Glucose concentration was determined by the glucose oxidase method.

Gut microbiota analysis (new-generation sequencing)

To assess gut microbiota, samples of $\sim$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 Illumina MiSeq 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 rdplclassifier 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.

Table 1

<table>
<thead>
<tr>
<th>Participants’ metabolic characteristics</th>
<th>ALA group (n = 12)</th>
<th>GLN group (n = 21)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Height (m)</td>
<td>1.62 ± 0.07</td>
<td>1.59 ± 0.06</td>
<td>0.15</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78.3 ± 16.0</td>
<td>78.4 ± 15.7</td>
<td>86.7 ± 15.2</td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>29.3 ± 4.4</td>
<td>29.4 ± 4.2</td>
<td>34.4 ± 5.9</td>
</tr>
<tr>
<td>WC (cm)</td>
<td>90.2 ± 9.2</td>
<td>90.2 ± 8.8</td>
<td>96.9 ± 11.9</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>84 ± 7.9</td>
<td>84 ± 8.9</td>
<td>79 ± 9.1</td>
</tr>
</tbody>
</table>

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.
Gut microbiota composition

Twelve phyla and 210 genera were observed in the gut microbiota of most volunteers. Certain groups of dominant bacteria were detected, namely Bacteroidetes and Firmicutes phyla, and Alistipes, Bacteroides, Megasphaera, Prevotella, Phascolarctobacterium, and Succinivibrio genera.

Figure 1 shows the relative proportions of the most relevant phyla at baseline and day 14 for both groups. After ALA treatment, we observed a decreasing trend of the Bacteroidetes phylum and an increasing trend in the relative proportion of Firmicutes and Actinobacteria, with an increase in the ratio of Firmicutes to Bacteroidetes (F/B) from 0.91 to 1.12 (Fig. 1A). In contrast, there was an increasing trend in the relative proportion of Bacteroidetes, a reduction of Firmicutes and Actinobacteria after 14 d of GLN treatment (Fig. 1B), with a decrease in the F/B ratio from 0.85 to 0.57. The proportion of Proteobacteria slightly increased in both groups.

For the comparison between the differences in phylum abundance (day 14 minus baseline) of the two groups, we observed that Actinobacteria and Firmicutes statistically differed between groups, most likely due to the steep decrease of these phyla in the GLN group. There were no significant differences between groups for the Acidobacteria, Bacteroidetes,

<table>
<thead>
<tr>
<th>Phylum</th>
<th>ALA Group (n = 12)</th>
<th>GLN Group (n = 21)</th>
<th>P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>kcal</td>
<td>1520 ± 409.3</td>
<td>1756 ± 482.5</td>
<td>0.24</td>
</tr>
<tr>
<td>CHO (%)</td>
<td>54.9 ± 9.6</td>
<td>50.5 ± 8.1</td>
<td>0.28</td>
</tr>
<tr>
<td>LIP (%)</td>
<td>30.6 ± 6.7</td>
<td>34.8 ± 10.7</td>
<td>0.28</td>
</tr>
<tr>
<td>PTN (%)</td>
<td>19.8 ± 18.5</td>
<td>19.9 ± 9.6</td>
<td>0.98</td>
</tr>
</tbody>
</table>

ALA, alanine; CHO, carbohydrate; GLN, L-glutamine; LIP, lipid; PTN, protein

Data were collected from a 24-h food record before and after 14 d of supplementation and from food diary of three consecutive days of groups ALA or GLN. Data are expressed as mean ± SD

* P-value from unpaired Student’s t test under normality and Wilcoxon Mann–Whitney test otherwise.
The results obtained using the GLRT with NB distributions showed that only the mean counts of Actinobacteria and Firmicutes at day 14 were significantly different from those of their baseline ($P = 0.006$ and $0.002$, respectively) for the GLN group. On the other hand, no significant difference was found, except for the Lentisphaerae phylum ($P = 0.043$) for the ALA group. These conclusions support the previous results in the sense that the mean counts of the GLN group that statistically differ (GLRT) from baseline to the 14 d, induce the significant differences between the groups found by the $t$ and Wilcoxon tests.

We found significant differences by using the GLRT in the abundance of Alastipes ($P = 0.02$), Bacteroides ($P < 0.001$), and Prevotella ($P < 0.001$) genera, for the Bacteroidetes phylum, and of Blautia ($P = 0.02$), Dialister ($P = 0.01$), Dorea ($P = 0.003$), Faecalibacterium ($P = 0.002$), Lachnospiraceae incertae sedis ($P = 0.01$), Phascolarctobacterium ($P = 0.02$), Roseburia ($P$, 0.04), and Ruminococcus ($P = 0.001$) genera that belong to the Firmicutes phylum, after GLN treatment. Thereby, these results corroborated the findings of the reduction on the entire Firmicutes phylum.

On the other hand, only Bacteroides ($P < 0.001$), Faecalibacterium ($P = 0.01$), and Pseudobutyrylbrio ($P = 0.01$) were significantly different after ALA treatment, but these differences were not enough to statistically alter the composition of any phylum.

### Discussion

In the present study, supplementation with GLN was able to alter the gut microbiota of overweight and obese individuals. It has been demonstrated that the gut microbiota of obese mice had a significantly higher proportion of Firmicutes and lower proportion of Bacteroidetes when compared with lean controls [16]. Similar results were observed in human [17,18] and animal [16,19–21] studies. A higher F/B ratio is considered a good biomarker for obesity [19]. In fact, one study found a reduction of F/B ratio in obese animals after weight loss [19]. Here, GLN
supplementation decreased the abundance of the Firmicutes phylum and reduced the F/B ratio from 0.85 to 0.57, whereas it increased from 0.91 to 1.12 in the ALA group. Thus, these findings suggest the effects of oral supplementation with GLN on gut microbiota are similar to those seen in weight loss. Although the age range of the volunteers was large (23–59 y) and aging may have an effect on intestinal microbiota [22], the results obtained in this study were statistically significant.

It is interesting to note that in our study there was no significant difference in Bacteroidetes between groups. This may be due to a similar reduction of Bacteroidetes abundance in both groups. However, the increase in proportion of Bacteroidetes due to a similar reduction of Bacteroidetes abundance in both groups decreasing, the number of Firmicutes in the GLN group decreased more sharply.

The significant reduction of the Firmicutes and Actinobacteria phyla observed by using the t and Wilcoxon tests was reinforced with the GLRT with NB distributions. All the significant differences in genera of bacteria observed in our study belong to the Firmicutes phylum. It has been demonstrated that Dialister genus decreased after bariatric surgery in obese patients [23]. Here, we observed a similar result with a reduction in Dialister genus after GLN supplementation, suggesting that GLN may modulate some bacteria genus in the gut.

Supplementation with GLN in obese rats was also associated with a reduction of the proinflammatory cytokines tumor necrosis factor-α and interleukin-6 in serum and in peripheral tissues [24,25], suggesting that GLN may have anti-inflammatory effects. Some bacteria genera are associated with gut inflammation. For example, increased levels of Veillonella are associated with higher levels of gut inflammation and the development of colitis and Crohn’s disease [26,27]. In our study, the number of bacteria from the Veillonella genus decreased after GLN supplementation, suggesting that GLN may have an anti-inflammatory effect, at least in part, due to the decrease of this genus. Additionally, increased abundance of the Prevotella genus has been described as a shield against inflammation and noninfectious diseases of the colon [28]. After GLN supplementation, but not after ALA, we observed an increase in Prevotella, suggesting that GLN may have a protective effect on the gut via modulation of bacteria.

In this study, we also detected a reduction of the Dorea and Pseudobutyryribidvo genera, but both have only recently been described as part of the microbiota and their importance to host physiology is not well known [29]. Nevertheless, the largest decreasing differences at the genus level did not statistically differ from GLN group to ALA group due to the large variability between individuals, but these contributed the most for the difference found in the overall phyllum.

We did not observe differences in body weight, WC, and fasting serum glucose between the two groups. It is important to note that individuals randomly selected to compose the ALA group had lower body weight and BMI than the group randomly selected to receive glutamine. The higher levels of body weight and BMI of patients who received glutamine may have influenced the lack of effect of GLN supplementation in these parameters.

However, the profile of the microbiota of individuals in the GLN group was similar to that found in patients participating in weight loss programs. Thus, it is possible that a study with a longer intervention period may result in metabolic changes.

Conclusion

Our study demonstrated that oral supplementation with L-glutamine for 14 d induced significant changes in the gut microbiota composition in overweight and obese adults. The finding that L-glutamine promotes changes in the gut microbiota composition provides support for the importance of some nutrients in modulating the intestinal bacterial profile. These changes resembled the weight loss programs established in the literature.

Acknowledgments

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References


