Brief report

Beneficial effects of Bifidobacterium lactis on lipid profile and cytokines in patients with metabolic syndrome: A randomized trial. Effects of probiotics on metabolic syndrome

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A B S T R A C T

Objective: Human studies have shown the beneficial effects of probiotic microorganisms on the parameters of metabolic syndrome (MetS) and other cardiovascular risks, but to our knowledge the effect of Bifidobacterium lactis has not yet been reported. The aim of this study was to evaluate the effect of consumption of milk containing the probiotic B. lactis HN019 on the classical parameters of MetS and other related cardiovascular risk factors.

Methods: Fifty-one patients with MetS were selected and divided into a control group (n = 25) and a probiotic group (n = 26). The probiotic group consumed fermented milk with probiotics over the course of 45 d. The effects of B. lactis on lipid profile, glucose metabolism, and proinflammatory cytokines (tumor necrosis factor-α and interleukin-6) were assessed in blood samples of the individuals at the baseline and after 45 d.

Results: Daily ingestion of 80 mL fermented milk with 2.72 × 10^10 colony-forming units of B. lactis HN019 showed significant reduction in body mass index (P = 0.017), total cholesterol (P = 0.009), and low-density lipoprotein (P = 0.008) compared with baseline and control group values. Furthermore, a significant decrease in tumor necrosis factor-α (P = 0.033) and interleukin-6 (P = 0.044) proinflammatory cytokines was observed.

Conclusion: These data showed potential effects of B. lactis HN019 in reducing obesity, blood lipids, and some inflammatory markers, which may reduce cardiovascular risk in patients with MetS.

Introduction

Metabolic syndrome (MetS) is a set of disorders, whose risk factors are atherogenic dyslipidemia (hypertriglyceridemia, high levels of apolipoprotein B, small, dense particles of low-density lipoprotein cholesterol, and low high-density lipoprotein cholesterol levels), hypertension, hyperglycemia, and a proinflammatory state [1].

Recent studies have associated the development of obesity in humans to the relative proportions of some major phyla of bacteria of the intestinal microbiota, such as Bacteroidetes, Firmicutes, and Actinobacteria, suggesting that the metabolic activity of these intestinal microbiota facilitates the extraction and/or

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storage of calories ingested [2,3]. Moreover, imbalance in this ecosystem can lead not only to obesity, as well as the development of insulin resistance [4–6].

In this context, probiotic microorganisms have demonstrated health-improving effects on the hosts. The Firmicutes and Actinobacteria include the Lactobacilli and Bifidobacteria, respectively, whose genera are the most used and often are related to these beneficial probiotic effects [7,8].

Although some studies have reported the beneficial effects of species of Lactobacilli in patients with MetS, we are not aware of any report on the effects of Bifidobacterium lactis in this condition and it is important to emphasize that the effects of probiotics are highly strain-dependent [9].

Therefore, the aim of this study was to evaluate the influence of fermented milk with B. lactis HNO19 on lipid profile, glucose metabolism, and cytokines in patients with MetS.

Participants and methods

The study was conducted according to the guidelines in the Declaration of Helsinki and approved by the Ethical Committee involving humans of the University of Londrina, Parana, Brazil, protocol number 185/2013. Patients were recruited between March and May 2013 in the city of Jataizinho, Parana State, Brazil. Inclusion criteria were being between ages 18 and 60 y, and meeting MetS diagnosis criteria, according to the National Cholesterol Education Program. Adult Treatment Panel III (NCEP/ATP III) [1]. Fifty-one patients with MetS were randomized by drawing and began to participate in the study Exclusion criteria involved patients with chronic diseases or individuals with use of drugs that interfere in the lipids and/or glycemic profile, antibiotics, and anti-inflammatory agents, vitamins and foods with probiotic microorganisms, besides lactose-intolerant individuals. The patients were instructed to maintain their usual diets, level of physical activity or other lifestyle factors throughout the intervention period.

Fifty-one patients with MetS agreed to participate in the study by written consent. The participants were divided into a control group or untreated (n = 25) and a probiotic group (n = 26). The diet of the probiotic groups included the daily ingestion of 80 mL of the probiotic milk containing on average 3.4 × 10^7 colony-forming units (CFU)/mL of B. animals ssp. lactis ssp. nov. HNO19.

The probiotic milk was produced and packaged aseptically at the Laboratory of Microbiology of the University of North Parana, according the current Brazilian legislation. The probiotic milk ready for consumption showed high probiotics counts with 3.4 × 10^7 CFU/mL, 0.38% acidity, pH 5.1, 2.80% protein, 7.54% carbohydrates, 0.0% fat, 12.08% total solids, and 0.48% ash that results in 33.00 calories per bottles. Additionally, the final product contained 0.01% (v/v) orange, vanilla, or both flavors. Pathogens were not detected, as required by legislation [10], thus the product was characterized as suitable for consumption and seven bottles of 80 mL were distributed weekly to each volunteer until they reached 45 d of intake. Patients were asked to drink one bottle every morning.

Anthropometric measurements and biochemical parameters were assessed at the beginning of the study (T0) and after 45 d (T45). Waist circumference (WC), body weight, and height were measured, and body mass index (BMI) was calculated (kg/m²). Three blood pressure measurements taken at 1-min intervals after the participant had been seated were recorded. The mean of these measurements was used.

After fasting for 12 h, serum or plasma samples were obtained and patients underwent the following laboratory blood analysis: glucose, total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and triacylglycerols (TGs). Levels were evaluated by a biochemical auto-analyzer (Dimension Dade AR Dade Behring, Deerfield, IL, USA), using Dade Behring® kits; plasma insulin level was determined by chemiluminescence microparticle immunoassay (Architect, Abbott Laboratory, Abbott Park, IL, USA). Homeostasis model assessment insulin resistance (HOMA-IR) was used as a surrogate measure of insulin sensitivity [11]. Tumor necrosis factor (TNF)-α levels were measured by a sandwich enzyme-linked immunosorbent assay (ELISA) using a commercial immunoassay ELISA (Ready-Set Go Set, e-Bioscience, San Diego, CA, USA).

The sample size was calculated to obtain a statistically significant result when changes of the parameters evaluated were at >10% level. This study was performed to obtain 80% statistic power. Mann-Whitney test was performed to compare differences between parameters of groups at baseline and differences across treatment groups (intergroup changes). Wilcoxon matched pairs test was performed to verify changes from baseline (intragroup changes). Data are presented as median (25% – 75%). A statistical analysis program (Graph Pad Prism version 4.0) was used for evaluations. Significance was set at P < 0.05.

Results

During the proposed study period, only one of the participants failed to join the group, claiming typical effects of lactose intolerance, although the recruitment survey excluded individuals with this characteristic.

There were no differences between control and probiotic groups with respect to age, sex, medication, and antihypertensive parameters at baseline.

No significant differences were observed intra- or intergroup for WC, systolic and diastolic blood pressure levels, TGs, HDL-C, glucose, insulin, and the HOMA (Table 1). The probiotic group showed significant decreases in BMI (P = 0.017), TC (P = 0.009), and LDL-C (P = 0.008) compared with baseline values. Regarding intergroup changes, there was a significant decrease (P < 0.05) in BMI, TC, and LDL-C verified after 45 d in the probiotic group compared with the control group. The control group had a significant increase in BMI and HDL-C, and a significant decrease in TC and LDL-C compared with the baseline values of the probiotic group (Table 1; P < 0.05).

Table 1

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Control (n = 25)</th>
<th>Probiotic (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T0</td>
<td>T45</td>
</tr>
<tr>
<td></td>
<td>P value</td>
<td>P value</td>
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<tr>
<td><strong>BMI (kg/m²)</strong></td>
<td>35.8 (33.4–44.5)</td>
<td>35.5 (32.2–40.7)</td>
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<tr>
<td></td>
<td>0.096</td>
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<tr>
<td><strong>WC (cm)</strong></td>
<td>107 (101–126)</td>
<td>109 (99.5–124.5)</td>
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<td></td>
<td>0.084</td>
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<td><strong>SBP (mm Hg)</strong></td>
<td>125 (112.8–139)</td>
<td>121 (110–131)</td>
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<tr>
<td></td>
<td>0.115</td>
<td></td>
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<tr>
<td><strong>DBP (mm Hg)</strong></td>
<td>77.5 (70–90)</td>
<td>72.5 (63.5–82.3)</td>
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<td>0.191</td>
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<tr>
<td><strong>TG (mg/dL)</strong></td>
<td>199 (103.3–251.3)</td>
<td>168.5 (113.5–221.3)</td>
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<tr>
<td></td>
<td>0.506</td>
<td></td>
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<tr>
<td><strong>TC (mg/dL)</strong></td>
<td>199 (166.5–208.5)</td>
<td>205 (173–220.5)</td>
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<tr>
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<td>0.067</td>
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<tr>
<td><strong>HDL-C (mg/dL)</strong></td>
<td>77.5 (70–90)</td>
<td>72.5 (63.5–82.3)</td>
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<tr>
<td></td>
<td>0.191</td>
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<tr>
<td><strong>LDL-C (mg/dL)</strong></td>
<td>117 (83–142)</td>
<td>115 (95–146)</td>
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<tr>
<td></td>
<td>0.320</td>
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<tr>
<td><strong>Glucose (mg/dL)</strong></td>
<td>98 (94–115.5)</td>
<td>91 (91–113.0)</td>
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<td></td>
<td>1.000</td>
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<tr>
<td><strong>Insulin (μU/mL)</strong></td>
<td>13.90 (9.93–17.78)</td>
<td>13.65 (9.46–21.78)</td>
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<td></td>
<td>1.07</td>
<td></td>
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<tr>
<td><strong>HOMA</strong></td>
<td>3.63 (2.09–4.44)</td>
<td>3.23 (2.33–5.55)</td>
</tr>
<tr>
<td></td>
<td>0.119</td>
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</table>

BMI, body mass index; DBP, diastolic blood pressure; HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, triacylglycerol; WC, waist circumference

Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (intergroup changes). Wilcoxon matched pairs test was performed to verify changes from baseline (intragroup changes). Data are expressed as median (25% – 75%).

- Differences of baseline groups, P < 0.05.
The cytokines TNF-α (P < 0.05) and interleukin (IL)-6 (P < 0.05) decreased after 45 d of probiotic intake compared with baseline values. Differences between treatment groups verified a statistically significant decrease in TNF-α and IL-6 (P < 0.05) when the probiotic group was compared with the control group (Fig. 1A, B).

Discussion

The main findings of the present study are related to the beneficial effects of B. lactis on BMI, lipid profile, and cytokine levels in patients with MetS. A recent report demonstrated that fermented milk with Lactobacillus plantarum decreased TC, glucose, and IL-6 levels. Meanwhile, there was a trend for decreases in LDL-C, although there were no significant differences in BMI [12].

Several studies have associated phyla and species of intestinal microbiota to obesity or weight loss in humans and high levels of bifidobacteria in the gastrointestinal tract are related to weight loss [4–6]. Although the fecal microbiota was not characterized in the present study, the high daily intake of B. lactis as allochthonous microbiota can positively affect the microbial balance [13] leading to an improvement of the lipid metabolism and weight loss. The mechanisms involved in body weight reduction are not clear, but studies have pointed to the reduction of adipocyte size, inhibition of adipogenesis, and suppression of energy intake [9,14].

The reduction of TC and LDL-C in the probiotic group corroborates others reports [9,15]. A meta-analysis of randomized controlled trials to evaluate the effects of probiotics observed a decrease in LDL and TC concentrations in individuals with normal, borderline high, and high cholesterol levels [15]. The mechanisms proposed for these effects are associated with the assimilation of cholesterol during bacterial multiplication or cholesterol binding to the surface of the cell wall of these bacteria, preventing the absorption by the intestinal tract into the bloodstream [16,17]. There are mechanisms related to the ability of some probiotic microorganisms to produce hydrolase that deconjugate bile acids. These compounds, when deconjugated, are absorbed less efficiently, resulting in increased excretion in the feces. Another hypothesis is the reduction of serum cholesterol as a function of lower lipid absorption, due to the coprecipitation of cholesterol together with the deconjugated bile acids, which are less effective in the solubilization and absorption of lipids from diet [18]. Additionally, the use of probiotics has been associated with increased levels of short-chain fatty acids (SCFA) such as propionate, which improve lipid metabolism by acting directly on the liver by inhibiting hydroxymethylglutaryl coenzyme A reductase, a rate-limiting step of the cholesterol synthesis pathway [19].

Studies evaluating probiotic intake and markers of inflammation are controversial. Some studies with Lactobacilli showed a decrease in inflammatory markers [20], whereas others did not report such findings [21,22]. In a recent study, the effects of a multispecies probiotic supplement containing L. acidophilus, L. casei, L. rhamnosus, L. bulgaricus, B. breve, B. longum, and Streptococcus thermophilus on high-sensitivity C-reactive protein (hs-CRP) in patients with diabetes have been reported. The authors found a significant decrease in serum hs-CRP levels [20]. Results from another study demonstrated the effects of L. plantarum on the classical parameters related to the MetS and other parameters related to cardiovascular risk [12]. The authors found a significant decrease in IL-6 between the control (P = 0.032) and the intervention (P = 0.001) groups. Therefore, it is difficult with these sparse data to support a strain specificity related to inflammatory status. The metabolic activity of gut microbiota is associated with the inflammatory responses originated from high-fat diets [14]. Bacterial lipopolysaccharides derived from intestinal microorganisms can act in this process triggering inflammatory response and consequently may predispose to the occurrence of MetS.

In this study, we observed the reduction on levels of TNF-α and IL-6, cytokines that have been widely studied and related to obesity. Fat accumulation caused by obesity provokes increased production of these adipokines, which stimulate the generation of hepatic acute-phase proteins, leading to proinflammatory condition associated with the development of obesity comorbidities as insulin resistance [23].

In this study, the small number of participants (N = 51) and the differences at the baseline values in some parameters between the groups limited the interpretation of the results. For example, decreases in TC, LDL-C, TNF-α, and IL-6 levels can be related to reduction in BMI. Nevertheless, the percentage of changes from baseline (intergroup differences) and intragroup differences in BMI, lipid profile, and cytokine levels were statistically significant (P < 0.05) and after 45 d (T45) of milk ingestion. Mann–Whitney test was performed to compare differences between the baseline values and across treatment groups (intergroup changes). Wilcoxon matched pairs test was performed to verify changes from baseline (intragroup changes). Data are expressed as median (25%–75%). *Differences of baseline values from groups (intergroup differences). *Percentage of change from baseline (P < 0.05).
differences attenuated this limitation. Additionally, the mechanisms that underlie the current findings are certainly needed, such as characterization of fecal microbiota and modifications of the genome. There were some strengths, however. To our knowledge, this is the first study to evaluate the effects of a species of *Bifidobacterium* on patients with MetS. Few studies have demonstrated the influence of probiotics on cytokines. We rigorously tried to ensure that the patients did not take any drug or presented any disease that could interfere with the results.

**Conclusion**

Data from the present study showed that regular consumption of *B. lactis* HN019 may contribute to the reduction of the characteristic parameters of MetS and obesity. Although the relationships between microbiota, obesity, diabetes, and MetS are increasingly evident, there remain many mechanisms that need to be explored. Currently, the main challenge is to identify bacteria that help to control obesity and related metabolic disorders.

**References**


