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A soy-based probiotic drink modulates the microbiota and reduces body weight gain in diet-induced obese mice

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ABSTRACT

This work investigates the effect of a soy-based probiotic drink (*Enterococcus faecium* CRL 183 and *Bifidobacterium longum* ATCC 15707) on the fecal microbiota composition, body weight and inflammatory parameters in diet-induced obese mice. The probiotic group had a lower body weight until 9th week of the study, reduced area and diameter of adipocytes, and showed a significant increase of IL-6 and IL-10 compared to the obesite non-treated group. The intake of a high-fat diet results in an increase of *Lactobacillus* spp. while the probiotic drink positively modulates the intestinal microbiota by maintaining the population of microorganisms belonging to the phylum *Bacteroidetes* and increases *Bifidobacterium* spp. Our study finds that the regular intake of this probiotic drink is able to reduce body weight gain and the size of adipocytes while modulating the fecal microbiota and the immune profile of animals, therefore acting in a beneficial manner in the control of obesity.

1. Introduction

In recent decades, several studies indicate changes related to the understanding of the risks and the genesis of various non-communicable diseases such as cardiovascular diseases, type 2 diabetes mellitus (T2DM) or non–insulin-dependent diabetes mellitus (NIDDM), insulin resistance, and obesity. The development of such diseases is related mainly to genetic predisposition, dietary habits and lifestyle to which individuals are subject. However, the etiology of these diseases is multifactorial, which complicates treatment and leads to high morbidity as well as mortality rates worldwide (Eid et al., 2017; WHO, 2014).

Among these non-communicable diseases, obesity – abnormal or excessive accumulation of body fat that causes a health risk to an individual (WHO, 2016) – has emerged in developed and developing countries (Bhurosy & Jeewon, 2014; Ng, Fleming, Robinson, Thomson, & Graetz, 2014). The etiology of obesity is represented by endogenous and exogenous factors, which can act at the same time or in isolation. These etiological factors contribute to an increase in adiposity that result in serious health conditions (Eid et al., 2017; Sabin, Werther & Kiess, 2011).

The intestinal microbiota composition is one of these etiological factors involved in the development of obesity and insulin resistance, as it influences fat storage, energy capture and it can triggers a systemic inflammation as well as metabolic disorders (Zhang et al., 2012). This complex ecosystem plays an important role in the proper function and homeostasis of the digestive system, and on the overall health of the human body (Koliada et al., 2017).

Evidence suggests that obese individuals exhibit a shift in the microbiota composition, displaying a higher population of the phylum *Firmicutes* with a minor population of *Bacteroidetes,* and *Firmicutes/Bacteroidetes* ratio tend to decrease with weight loss. This imbalance in the composition of the intestinal microbiota, known as dysbiosis, seems to promotes an increase in energy absorption capacity from the diet,

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modulating the individual's immune response and leading to a state of obesity (Koliada et al., 2017; Krajmalnik-Brown, Ilhan, Kang, & DiBaise, 2012; Sweeney & Morton, 2013). Some studies indicate that a shift in diversity of intestinal microbiota and specific microrganism genera are also associated with the development of obesity (Carlucci, Petrof, & Allen-Vercoe, 2016; Le Chatelier et al., 2013; Million et al., 2012; Million, Lagier, Yahav, & Paul, 2013).

Consequently, the ingestion of probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit on the host" (FAO/WHO, 2002; Hill et al., 2014), has been used as a strategy to positively modulate the microbiota and thereby reduce the risk of, or aid in the treatment of different diseases, including obesity (Kobyliak et al., 2016). Some species of *Lactobacillus* spp. benefit obese mice in addition to humans, demonstrating an influence on both lipid metabolism and body weight. A similar effect was verified when *Bifidobacterium* spp. strains were incorporated into the regular diet (Alard et al. 2016; Marchesi et al., 2016).

Previous studies showed that a soy-based product fermented with *Enterococcus faecium* CRL 183 and *Lactobacillus helveticus* 416 is capable of positively modulating inflammatory markers, the intestinal microbiota, and adipocyte circumference as well as deposits of body fat (Cavallini et al., 2011; Manzoni et al., 2005). However, the relationship between the ingestion of the this specific probiotic product, modulation of microbiota, and obesity has not yet been studied.

Considering the above, the aim of the present study was to verify the effect of a soy-based product, fermented with *E. faecium* CRL 183 and *L. helveticus* 416 with the addition of *Bifidobacterium longum* ATCC 15707, on the composition of fecal microbiota in mice fed a high-fat diet and its relationship with inflammatory parameters and body weight variation.

2. Material and methods

2.1. Material

The soy based probiotic product was fermented by a mixed inoculum of *E. faecium* CRL 183 (Center of reference to *Lactobacillus* - CERELA, Tucumán, Argentina) and *L. helveticus* 416 (Institute of food technology - ITAL, Campinas, SP, Brazil) with the addition of *B. longum* ATCC 15707 (American Type Culture Collection, USA).

2.2. Methods

2.2.1. Probiotic and placebo products

Probiotic and respective placebo products were obtained from UniverSoja – a unit that develops and produces soy products at the Faculty of Pharmaceutical Sciences, UNESP-Araraquara, Brazil. The fermented product was obtained according to the methodology proposed by Rossi, Vendramini, Carlos, Pei, and de Valdez (1999), with modifications (Celiberto et al., 2017), using the starting cultures *E. faecium* CRL 183 (probiotic - 1.5% v/v) and *L. helveticus* 416 (technological purposes - 1.5% v/v). The fermentation was carried out at 37 °C and when the product reached a pH of 4.5, the strain of *B. longum* ATCC 15707 (probiotic) was added in sufficient amount to achieve 8 logCFU ml^{-1} in the final product. The strains were propagated in milk medium (10% of skimmed-milk powder, 1% glucose, 0.5% yeast extract) overnight at 37 °C before being used in the preparation of the products. The viability of each strain was determined immediately after the preparation of the probiotic product (T0) and after seven days of storage at 5 °C (T7). For CFU counts, *L. helveticus* 416, *E. faecium* CRL 183 and *B. longum* ATCC 15707 were plated in Lactobacilli Man Rogosa Sharpe agar – MRS medium (Difco, France), M17 agar (Difco, France), and BIM-25 agar (Reinforced Clostridium agar Difco, France - with the addition of nalidixic acid, polymyxin B sulfate, kanamycin sulfate, iodoacetic acid and triphenyl tetrazolium chloride), respectively. The plates of MRS and M17 were incubated under aerobic conditions for 48 h at 37 °C and BIM-25 plates were incubated under anaerobic conditions for 72 h at 37 °C. The placebo product (unfermented) had identical composition compared to fermented product, without the bacterial cultures. The product was acidified by adding lactic acid food grade (Purac Phytochemical, Brazil) in sufficient amount to reach a pH of 4.5, similar to the fermented product. The products were manufactured weekly and stored at 5 °C ± 1 °C until their administration in the experiment.

2.2.2. Study in animal model

Adult Swiss male mice (Unib: SW) (8 weeks old), Specific Pathogen Free (SPF), were purchased from the Multidisciplinary Centre for Biological Research in the area of science in laboratory animals (CEMIB - UNICAMP, Brazil). The experimental procedures were performed at São Paulo State University (UNESP - Araraquara, Brazil) and the protocol employed was approved by the Sao Paulo State University's Animal Care Committee (CEUA) under the protocol 05/2016.

Animals were housed in polypropylene cages on ventilated shelf (Alesco E-520, Brazil), at 22 °C ± 2 °C with controlled photoperiod (12 h light/12 h dark). During the acclimatization period the animals received only standard diet specific to rodents (Presence® - Labina), with nutritional composition according to specifications of the American Institute of Nutrition (AIN-93M) (Reeves, Nielsen & Fahey, 1993) - total energy value (TEV): 3.6 Kcal/g; protein: 26% of TEV; lipid: 11% of TEV; carbohydrate: 63% Kcal of TEV - and autoclaved water *ad libitum*. After this period, the animals were randomly assigned into four groups (n = 10):

- Control (C): animals that received standard diet;
- Obese (OB): animals that received high-fat diet (HFD);
- Obese + fermented probiotics (OBF): animals that received HFD plus the probiotic fermented product;
- Obese + placebo (OBP): animals that received HFD plus the placebo product (unfermented and without addition of probiotic cultures).

The control group (C) continued to receive the standard diet, while the OB, OBF and OBP groups began to receive HFD (TEV: 5.15 Kcal/g; protein: 14.48% of TEV; lipids: 61.01% of TEV; carbohydrates: 24.51% of TEV), from Prag Bioscience ® Solutions (Jaú, Brazil) (Table 1). The composition of the diet has been adapted from Lenquiste et al. (2015), taking into consideration what is required by AIN-93M (Reeves et al., 1993), with additional animal fat to increase the caloric content of the diet and induce weight gain in the animals. All groups received the diets *ad libitum* during the 70 days of the experimental protocol. Water and food intake as well as body weight were monitored daily before the

Table 1
Nutritional composition of high-fat diet administered to animals under study.

Ingredients	High-fat diet (HFD)		
	Quantity (%)	Kcal/g	Kcal (%)
Corn starch	4.43	0.18	3.44
Ground soybean meal	41.00	1.32	25.75
Dextrinizado starch	5.00	0.20	3.89
Sucrose	8.00	0.32	6.22
Lard	30.20	2.72	52.80
Soybean oil	4.00	0.36	6.99
Microcrystalline cellulose	2.54	–	–
L-cystine	0.18	0.01	0.14
Choline bitartrate	0.15	–	–
Butylated hydroxytoluene	0.00	–	–
Mineral mix AIN 93	3.50	–	–
Vitamin mix AIN 93	1.00	0.04	0.78
Total	100.00	5.15	100.00

High-fat diet (HFD): 18.63% of proteins (14.48% of VET); 34.9% of lipids (61.01% of VET) e 31.55% of carbohydrates (24.51% do VET).
Kcal/g: kilocalorie/gram.
Composition of the diet adapted from Lenquiste et al. (2015).