

Changes in weight and body fat after use of tetracycline and *Lactobacillus gasseri* in rats

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Recent studies have shown a role of intestinal microbiota in obesity. The consumption of antibiotics in the last 70 years has led to changes in intestinal microbiota, which has led to weight gain and body fat accumulation. To evaluate the possibility of weight gain induced by antibiotics and the possible protective effect of probiotics, we divided 45 animals (*Rattus norvegicus*) into groups and administered the following treatments over two weeks: tetracycline, tetracycline + *Lactobacillus gasseri*, and NaCl. The animals were weighed over the course of 8 weeks, and at the end of the treatment period, they were measured and subjected to bioelectrical impedance analysis. The results show that the group receiving tetracycline alone had a higher body mass index ($p=0.030$), a greater Lee index ($p=0.008$), and a lower body water percentage than the control group, indicating a greater accumulation of body fat. The group receiving the probiotics with tetracycline presented similar results to the control group, indicating a possible protective effect of body fat accumulation in the group receiving tetracycline alone. The results show that tetracycline increased the concentration of body fat, and the use of probiotics was associated with an ability to protect the animals from the pro-obesity effect.

Uniterms: Antimicrobials/evaluation. Obesity/tests. Gut microbiota/tests. Tetracyclines.

INTRODUCTION

Obesity is currently one of the greatest global public health challenges, and the number of obese people has risen dramatically, especially in Western countries (Riley *et al.*, 2013). In the United States alone, the number of overweight people has doubled from an average of 15.1% of the population in 1976–1980 to 31.2% in 2001–2004 (Christian, Rashad, 2009). Obesity is associated with other grave medical conditions, such as cancer, diabetes, hypertension, and cardiovascular disease. Diet and physical inactivity are certainly important causes of obesity, but they are not the only ones. The multifactorial etiology of obesity (Newnham *et al.*, 2009; Riley *et al.*, 2013) also includes changes in the human microbiota (Musso, Gambino, Cassader, 2011; Moreira *et al.*, 2012; Cho *et al.*, 2012; Million *et al.*, 2013; Riley *et al.*, 2013; Martinez, 2014).

The human gut microbiota has about 100 trillion bacterial cells that predominantly belong to four bacterial phyla: Proteobacteria, Actinobacteria, Firmicutes, and Bacteroidetes. The latter two represent about 90% of the organisms found in the gut microbiota (Tilg, Kaser, 2011; Riley *et al.*, 2013). There are 10 times more intestinal bacterial cells than the cells that comprise a human adult. This large number of microorganisms is associated with numerous functions that are developed and mediated by gut microbiota and have led some authors to refer to the gut microbiota as another human organ (Quigley, 2013; Riley *et al.*, 2013; Lankelma *et al.*, 2014; Putignani *et al.*, 2014).

Although relatively stable, the gut microbiota is affected by age, gender, geography, diet, and environmental factors, such as exposure to antibiotics, especially in children and newborns (Backhed *et al.*, 2005; Agans *et al.*, 2011; Vael *et al.*, 2011; Grzeskowiak *et al.*, 2012; Markle *et al.*, 2013; Trasande *et al.*, 2013). There are numerous studies related to gut microbiota changes (dysbiosis) caused by antibiotics and probiotics, including weight modifications in humans and animals (Ley *et al.*, 2006; Turnbaugh *et al.*, 2008; Xu *et al.*, 2012; Bibiloni, Pons,

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Tur, 2013). The relationship between Bacteroidetes and Firmicutes seems to be a major factor in the increase or decrease of energy gain, although there is no consensus on the role of each microorganism in the development of obesity or weight loss (Collado *et al.*, 2008; Li *et al.*, 2008; Turnbaugh *et al.*, 2009; Schwartz *et al.*, 2010).

In the last 70 years, we have been exposed to large amounts of antibiotics as therapies against infections (Van Boeckel *et al.*, 2014), in antibiotic-contaminated food (Anderson *et al.*, 2003) 2003, or in the large amounts dumped into the environment (Sarmah Meyer, Boxall, 2006). This increased exposure, especially in children, may be responsible for the recent obesity epidemic. Studies show that increases in body weight caused by microbial antibiotics is becoming more frequent, particularly when the antibiotic has a broad spectrum of activity. Alternatively, the use of probiotics has been associated with weight loss and could presumably protect against the effect caused by antibiotics in the microbiota (Haight, Pierce, 1955; Saiman *et al.*, 2010; Thuny *et al.*, 2010; Saiman *et al.*, 2012; Trasande *et al.*, 2013).

In a recent meta-analysis involving 88 studies on the effect of *Lactobacillus* species on the weight of animals and humans, Million *et al.* (2012) showed that some species were associated with weight gain (*L. acidophilus*, *L. ingluviei*, and *L. fermentum*), while others were associated with weight loss (*L. plantarum* and *L. gasseri*). To discover more information about this phenomenon and to search for possible treatments with a combination of probiotics, this study evaluated the influence of tetracycline with or without a probiotic on the weight and body composition of rats.

METHODS

Animal care: We used 45 male rats (*Rattus norvegicus* Wistar) that were 15 days old. The animals were housed in the animal house at the University of Sorocaba, where they stayed for a 10-day adaptation period before the experimental phase. They were fed with standard rodent chow (Presence®) and water *ad libitum*. The project was approved by the Ethics Committee on Animal Research of the University of Sorocaba (n.010/2013), and it was conducted in accordance with the Brazilian Regulations for Animal Experimentation. The animals were housed in cages holding 3 rats each with free access to chow and water. They were exposed to a cycle of 12 hours of light and 12 hours of dark and kept at 22 °C.

Treatment: The animals were divided into 3 groups of 15 animals each: the TETRA Group, the TETRA+LG Group, and the CONTR Group. The treatments were given

to each group once daily by an oral route using a gavage needle. TETRA was given a tetracycline dose of 75 mg/kg/day; TETRA+LG was given a dose of tetracycline of 75 mg/kg/day followed 2 hours later by treatment with a 0.1-mL suspension containing 10⁹ cfu of *L. gasseri* (ATCC 33233). The CONTR group was given 0.1 mL of NaCl solution (0.9%). The treatments were given for two weeks on days 0, 2, 4, 7, 9, and 11. The *L. gasseri* was dissolved in a saline solution containing 0.9% NaCl.

Probiotic: *L. gasseri* was obtained in a lyophilized form from the André Tosello Foundation (ATCC 33233 (American Type Culture Collection)) and maintained and grown in MRS BROTH (DE MAN, Rogosa, SHARPE).

Weight gain and body composition

Animal weight (AW): The rats were weighed weekly for 8 weeks on days 0, 7, 14, 21, 28, 35, 42, 49, and 56. To assess body composition, the rats were measured and subjected to bioelectrical impedance analysis.

Body measurements: To measure the bodies of the animals, they were anesthetized with sodium pentobarbital (30 mg/kg) and measured as described previously. (Novelli *et al.*, 2007) Abdominal circumference (AC) was measured immediately anterior to the forefoot, thoracic circumference (TC) was measured immediately behind the foreleg, and body length (BL) was the nose–anus length. We used a non-elastic strap with an accuracy of 0.1 cm.

Bioelectrical impedance: Tetrapolar bioelectrical impedance analysis (TBIA) was performed according to a previously described procedure (Hall, Lukaski, Marchello, 1989) Whole-body reactance (WBXc) and whole-body resistance (WBR) were measured using a tetrapolar phase-sensitive bioelectrical impedance analyzer that introduced a 42-µA current at 50 kHz (Bioelectrical Body Composition Analyzer, Model: Quantum II). The anesthetized animals were placed in a prone position on a nonconductive surface. The hair was removed, and four electrodes (two sources and two detectors) made from hypodermic needles were placed as described previously (Hall, Lukaski, Marchello, 1989; Yokoi *et al.*, 2001). Electrode 1 (a source) was placed at the anterior edge of the orbit, electrode 2 (a source) was placed 4 cm from the base of the tail, electrode 3 (a detector) was placed at the anterior opening of the pinna, and electrode 4 (a detector) was placed at the midpelvis.

We evaluated the following parameters for all animals using the data obtained from the TBIA (WBXc and WBR), the body measurements (AC, TC, and BL), and the animal weights (AW):

- The body mass index (BMI) was calculated by divid-

ing the weight of the animal in grams (AW) by the square of its length in centimeters (BL) (Novelli *et al.*, 2007).

- The Lee index was determined by dividing the cubic root of the animal weight in grams (AW) by the length in centimeters (BL) (Bernardis, 1970).
- The AC/TC ratio was determined by dividing the AC in centimeters by the TC in centimeters.
- The total body water in grams (TBW) was estimated using a previously described empirical formula (Hall, Lukaski, Marchello, 1989), $TBW = 15.47 + 97.44 BL^2/WBR$, where BL is the body length in centimeters and WBR is in Ω and obtained from the TBIA.
- The percentage of body water (% BW) was calculated by dividing the TBW in grams by the animal weight in grams (AW) and multiplying the result by 100.

Statistical analysis

All measurements were taken twice. The statistical significance between the three groups was calculated using a two-way analysis of variance (ANOVA), followed by

the Tukey-Kramer test. A difference between groups was considered statistically significant when the p value was <0.05 .

RESULTS

Weight Gain: The rats were weighed weekly. Table I shows the initial weight average (day zero), the final weight medians (day 56), the average weight gain, and the standard deviation. We observed no difference in weight gain among the three groups.

The data in Figure 1 show that there was no variation in the weekly weight of the animals in any of the groups. The animals in all groups showed a fairly homogenous weight gain, with higher gain between the third and fourth weeks after the beginning of the experiment. Table II shows the results of the bioelectrical impedance for the WBXc and the WBR data. There was no difference between any groups tested in the bioelectrical impedance parameters evaluated.

Table III shows the results obtained from the body measurements and TBIA. The BMI was significantly higher in the TETRA group than in the CONTR group ($p=0.0302$). The Lee index was significantly higher in the

TABLE I - Initial weight, final weight, and weight gain of animals [mean (\pm SD)] for each group

	TETRA	TETRA+LG	CONTR	p
Initial weight (g)	74.05 (\pm 5.11)a	77.53 (\pm 7.94)a	76.33 (\pm 3.84)a	0.7161
Final weight (g)	336.06 (\pm 11.34)a	336.53 (\pm 14.78)a	332.46 (\pm 10.09)a	0.8835
Weight gain (g)	261.13 (\pm 7.38)a	259.00 (\pm 11.66)a	256.13 (\pm 7.63)a	0.7538

TETRA – Tetracycline group (75 mg/kg/day); TETRA+LG – Tetracycline plus *L.gasseri* group (75 mg/Kg/day plus 10^9 cfu); CONTR – Control group; Different superscripted letters, in same row, indicate statistical significance $p<0.05$.

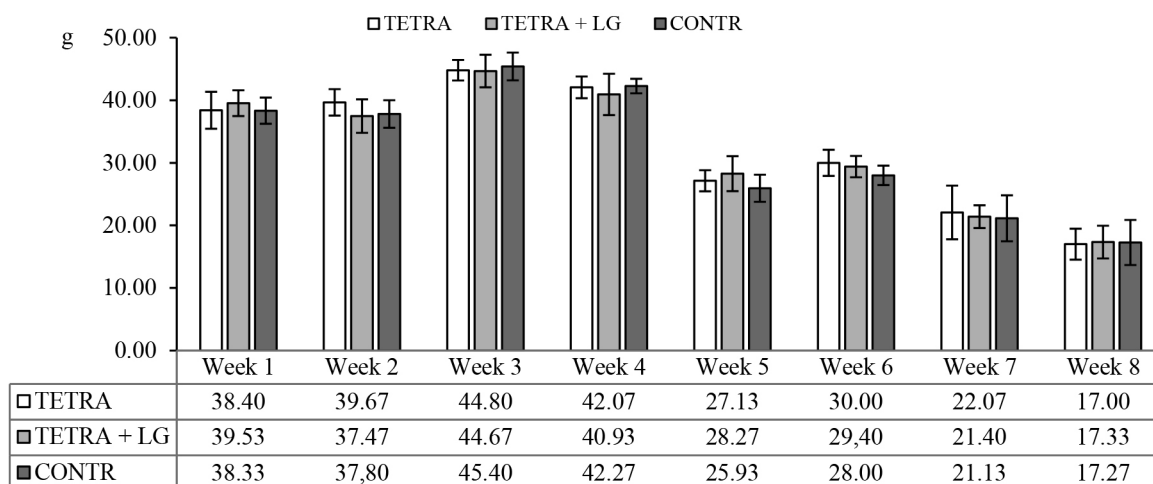


FIGURE 1 - Weekly average weight gain (g) and standard deviation.

TABLE II - Bioelectrical impedance: whole body reactance (WBXc) and whole body resistance (WBR) for the three groups

	TETRA	TETRA+LG	CONTR	p
WBXc	26.67 (\pm 2.35) ^a	23.00 (\pm 2.38) ^a	24.40 (\pm 2.30) ^a	0.1104
WBR	227.03 (\pm 7.00) ^a	216.67 (\pm 10.89) ^a	212.87 (\pm 9.74) ^a	0.1067

WBXc – Whole-body reactance; WBR - Whole-body resistance; TETRA – Tetracycline group (75 mg/kg/day); TETRA+LG – Tetracycline plus *L.gasseri* group (75 mg/kg/day plus 10^9 cfu); CONTR – Control group; Different superscripted letters, in same row, indicate statistical significance $p < 0.05$.

TABLE III - BMI, Lee index, AC/TC ratio, TBW, and BW in all groups

	TETRA	TETRA+LG	CONTR	p
BMI (g/cm ²)	0.615 (\pm 0.029) ^a	0.576 (\pm 0.031) ^{a,b}	0.562 (\pm 0.019) ^b	0.0302
Lee index	0.297 (\pm 0.006) ^a	0.287 (\pm 0.006) ^b	0.284 (\pm 0.008) ^b	0.0082
AC/TC ratio	1.16 (\pm 0.032) ^a	1.121 (\pm 0.035) ^{a,b}	1.110 (\pm 0.025) ^b	0.0144
TBW (g)	252.1 (\pm 13.70) ^a	280.7 (\pm 12.16) ^b	288.8 (\pm 15.62) ^b	0.0024
BW (%)	76.42 (\pm 4.25) ^a	84.50 (\pm 4.41) ^b	87.29 (\pm 5.47) ^b	0.0052

BMI – Body Mass Index; AC/TC ratio – Abdominal Circumference / Thoracic Circumference Ratio; TBW (g) – Total body water in grams; BW (%) – Percentage of body water; TETRA – Tetracycline group (75 mg/kg/day); TETRA+LG – Tetracycline plus *L.gasseri* group (75 mg/kg/day plus 10^9 cfu); CONTR – Control group; Different superscripted letters, in same row, indicate statistical significance $p < 0.05$.

TETRA group (which received only tetracycline) than the TETRA+LG and CONTR groups ($p = 0.0082$), but there was no difference between the TETRA+LG and CONTR groups. The results for the AC/TC ratio were similar to BMI, showing a difference between only the TETRA group and the CONTR group ($p = 0.0144$).

The TBW and the BW results showed that the TETRA group, which received only the antibiotic, had a lower amount of body water (252.1 g) and the lowest percentage of body water (76.42%), indicating a greater amount of fat ($p = 0.0024$ and $p = 0.0052$). The administration of *L. gasseri* associated with tetracycline in the TETRA+LG group was able to protect against the accumulation of body fat that occurred in the TETRA animals with similar performance to the CONTR group.

DISCUSSION

Data from this study show that the animals treated with tetracycline alone had significantly lower body water percentage than the other groups, although the weight gain was equal for all groups. Other studies have shown similar results. Cho used a different methodology and showed that giving animals antibiotics increased their body fat, despite not finding differences in weight (Cho *et al.*, 2012). There is ample evidence showing that the use of antibiotics may be causing weight gain in humans, especially when administered to children (Perrini, 1951;

Macdougall, 1957; Garly *et al.*, 2006; Southern *et al.*, 2012; Trasande *et al.*, 2013).

This adverse effect should not limit the use of antibiotics. During the first years of life when respiratory infections most often occur, antibiotic use is more frequent and necessary (Razon *et al.*, 2005; Del Fiol *et al.*, 2013). However, the use of antibiotics in this age group is a great concern because the microbiotas are more susceptible to changes (dysbiosis) at this time (Li, Wang, Donovan 2014; Voreades, Kozil, Weir, 2014). Therefore, it is very important to understand that probiotics could provide protection against the adverse effects of antibiotics and protect the microflora of these children during antibacterial therapy. *L. gasseri* could be used as an alternative to protect against dysbiosis and the resulting weight gain.

Studies of *L. gasseri* in humans have shown decreases in abdominal adiposity, body weight, and waist and hip circumferences after oral use of probiotics (Kadooka *et al.*, 2010; Jung *et al.*, 2013). Several studies show that the use of this microorganism in rats decreased visceral fat by enhancing lipid excretion (Yonejima, Ushida, Mori, 2013), reduced adipocyte size (Sato *et al.*, 2008; Hamad *et al.*, 2009), and reduced body weight (Kang Yun, Park, 2010; Kang *et al.*, 2013).

The role of the microbiota in obesity and interference by antibiotics are widely documented. This study has demonstrated that tetracycline increases the fat mass of treated animals, as in other studies, and that probiotics (*L.*

gasseri in this case) could protect the microbiota while preventing or decreasing fat accumulation after the use of antibiotics. Other studies are necessary to elucidate the mechanism by which some probiotics interfere with weight loss or gain in order to combat malnutrition and obesity, which are two major problems afflicting global health.

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