Obesity and the Use of Antibiotics and Probiotics in Rats

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Key Words

Antibiotics · Obesity · Probiotics

Abstract

Background: Obesity has become a major public health challenge in recent years. Recent studies suggest that alterations of the gut microbiota by antibiotics could play an important role in obesity. **Methods:** We investigated this topic using 60 Wistar rats, which were divided into 3 experimental groups: rats treated with amoxicillin, rats treated with amoxicillin plus *Saccharomyces boulardii* and controls. Treatments were administered over the course of 2 weeks. Tetrapolar bioelectric impedance analysis and anthropometric evaluations were conducted. **Results:** The body mass index was significantly lower for the animals in the control group ($p = 0.034$). The same result was observed for the Lee index: the control group had a lower index than the 2 groups that received antibiotic treatment ($p = 0.0019$). The total body water data demonstrated that the control group had the greatest amount of body water (279.1 g, $p = 0.0243$). **Conclusions:** The groups treated with the antibiotic exhibited a greater accumulation of body fat than the control group.

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Introduction

Obesity has become a major public health challenge in recent years and is now being treated as a true pandemic, particularly in Western countries [1]. In addition to the difficulties in locomotion that are caused by obesity, the severe medical problems that accompany this disease include diabetes, hypertension and dyslipidemia [2].

The multifactorial etiology of obesity is linked to an individual's unique factors, such as genetic background and physical activity, and to environmental and cultural factors associated with modern life, such as high-calorie food, high-fat diets and the gradual replacement of human activities by mechanized activities [2, 3].

To better understand the role of the gut microbiota in this disease, scientists have recently begun to evaluate the possibility that antibiotic-induced changes in the intestinal microbiota could interfere directly with the body's metabolism and energy balance [1, 2, 4, 5]. Some authors have referred to this change in intestinal microbiota as dysbiosis, and many studies have attributed these changes to antibiotics. The emergence of dysbiosis may be a possible explanation for the occurrence of a worldwide epidemic of obesity [2, 6–8].

Since the 1940s, antibiotics have been added in low doses to animal feed to increase weight gain. This process increases the efficiency of feeding poultry, pigs and cattle

[9–12], particularly when the antibiotics are administered in the first days of an animal's life [7].

Babies acquire their microbiota in early life via contact with contaminated surfaces, such as maternal vaginal surfaces, fecal microbiota [13] and other family members. The composition of the microbiota varies and is highly susceptible to changes during childhood. A level of stability is acquired in adulthood and is affected by age [14, 15], sex [16], geography [17], diet [18], pathogenic bacteria [19] and environmental factors, such as early exposure to antibiotics [7].

The administration of antibiotics to children during childhood and associated alterations in gut microbiota (i.e. dysbiosis) could be responsible for the significant increases observed in the indicators of childhood and adult obesity [20]. This phenomenon has been the focus of numerous studies that demonstrated that a significant direct relationship exists between the use of antibiotics in childhood and the emergence of obesity [2, 6–8, 18, 21].

To obtain more information and to search for potential probiotic-based treatments, this study evaluated the interference of the administration of amoxicillin in the presence and absence of probiotics with the weight and body composition of rats.

Methods

Animal Care

Sixty male Wistar rats with an age of 12 days were used. The animals were received at the vivarium of the University of Sorocaba and were allowed to adapt to the experimental conditions for 10 days. A standard laboratory rodent diet (Presença®) and water were provided ad libitum.

The project was approved by the University of Sorocaba's Ethics Committee on Animal Research (No. 010/2013) and was conducted in accordance with the Brazilian regulations for animal experimentation. The animals were housed in cages under an alternating 12-hour light/dark cycle. The room temperature was maintained at 22°C.

The 60 animals were divided into 3 experimental groups: group AMOX received amoxicillin ($n = 20$), group AMOX + SB received amoxicillin plus *Saccharomyces boulardii* ($n = 20$), and group CONTR contained the controls ($n = 20$).

Treatments

Group AMOX received 150 mg/kg of amoxicillin per os as a single daily dose. Group AMOX + SB received 150 mg/kg of amoxicillin per os and 0.1 ml of a suspension of *S. boulardii* (2.8×10^6 CFU) 2 h later. Both treatments were administered as a single daily dose. Group CONTR received 0.1 ml of 0.9% NaCl as a single daily dose.

The treatments were administered over the course of 2 weeks on days 0, 2, 4, 7, 9 and 11, with a total of 6 administrations for each group. The lyophilized *S. boulardii* cells were dissolved in saline (0.9% NaCl) for their administration to the animals.

Weight Gain Evaluation

To evaluate weight gain, the animals were weighed weekly for 8 weeks, on days 0, 7, 14, 21, 28, 35, 42, 49 and 56.

Assessment of Body Composition

To assess the body composition, the animals underwent anthropometry and bioelectrical impedance testing.

Bioelectrical Impedance

Tetrapolar bioelectric impedance analysis (TBIA) was performed according to the procedure described by Hall et al. [22]. Whole body reactance and whole body resistance (WBR) were measured using a tetrapolar phase-sensitive bioelectrical impedance analyzer that introduced a 425- μ A current at 50 kHz (Bioelectrical Body Composition Analyzer, model Quantum II). The animals were anesthetized with an intraperitoneal injection of pentobarbital (30 mg/kg) and placed in the prone position on a non-conductive plastic surface. Their hair was removed, and 4 electrodes (i.e. 2 sources and 2 detectors) made from hypodermic needles were placed as described by Hall et al. [22] and Yokoi et al. [23]. Electrode 1 (i.e. a source) was placed at the anterior edge of the orbit, electrode 2 (i.e. a source) was placed 4 cm from the base of the tail, electrode 3 (i.e. a detector) was placed at the anterior opening of the pinna, and electrode 4 (i.e. a detector) was placed at the mid pelvis of the rats.

Anthropometric Determinations

For the anthropometric evaluation of the animals, the following measurements were made, as described by Novelli et al. [24]: the abdominal circumference (AC) was measured immediately anterior to the forefoot, the thoracic circumference (TC) was measured immediately behind the foreleg, and the body length (BL) was measured as the nose-anus length. The anthropometric measurements were made using a plastic, nonextensible measuring tape, with an accuracy of 0.1 cm. All measurements were made on anesthetized animals after the TBIA.

Using the data obtained from the TBIA (i.e. whole body reactance and WBR) and from anthropometry (i.e. AC, TC and BL) and the animal weights (AW), we evaluated the following parameters for all animals:

- the body mass index (BMI) was calculated by dividing the AW in grams by the square of its BL in centimeters [24];
- the Lee index was determined by dividing the cubic root of the AW in grams by the BL in centimeters [25];
- the AC/TC ratio was determined by dividing the AC in centimeters by the TC in centimeters;
- the total body water (TBW) in grams was estimated as follows, using the empirical formula described by Hall et al. [22]: $TBW = 15.47 + 97.44 \text{ BL}^2 / \text{WBR}$, where the BL is in centimeters and the WBR is in ohms from the TBIA;
- the percentage of body water was calculated by dividing the TBW in grams by the AW in grams. The result was multiplied by 100 to obtain the percentage.

Statistical Analysis

All measurements were performed in duplicate. The statistical significance of differences among the 3 groups was evaluated using 1-way analysis of variance followed by the Tukey-Kramer test. A difference between the groups was considered statistically significant when the p value was <0.05 .

Fig. 1. Mean weekly weight gain in grams for the AMOX, AMOX + SB and CONTR groups.

Results

Weight Gain

The animals were weighed weekly. Table 1 shows the mean initial weight (i.e. day 0), the mean final weight (i.e. day 56) and the mean weight gain (in grams) of the studied groups. Although we observed a higher rate of weight gain in the group AMOX + SB, this result was not statistically significant ($p = 0.13$). Figure 1 shows the mean weekly weight gain for all of the groups. Interestingly, the 2 groups that received antibiotic treatment (i.e. AMOX and AMOX + SB) gained less weight in the first 2 weeks than the CONTR group. However, this difference was not statistically significant ($p = 0.504$). This tendency was reversed after the third week, when the CONTR group began to gain less weight. Interestingly, of all of the weeks studied, a significant difference in the weekly weight gain of the animals was observed only in the last week (i.e. week 8), when the CONTR group gained less weight (15.15 g) than the groups receiving amoxicillin ($p = 0.0134$).

Body Composition

Table 2 shows the results of the TBIA. No differences were observed among the groups. The data presented in table 3 are the results obtained from anthropometry and TBIA. The BMI was significantly lower for the animals in the CONTR group. The same result was observed for the Lee index, for which the group that received no antibiotic treatment (i.e. CONTR) exhibited a lower value ($p = 0.0019$) than the 2 groups that received the antibiotic (i.e. AMOX and AMOX + SB).

Table 1. Mean initial weight, final weight and weight gain of the animals according to treatment group

	AMOX	AMOX + SB	CONTR
Initial weight, g	101.05±9.68 ^a	103.75±8.09 ^a	102.15±8.72 ^a
Final weight, g	381.00±6.80 ^a	396.75±8.71 ^a	375.10±8.47 ^a
Weight gain, g	279.95±26.16 ^a	293.00±35.25 ^a	272.95±32.86 ^a

^a Superscript letters indicate the absence of a statistical significance of $p > 0.05$.

Table 2. TBIA data for the AMOX, AMOX + SB and CONTR groups

	AMOX	AMOX + SB	CONTR	p
Whole body reactance	25.77±5.41 ^a	21.94±8.82 ^a	25.30±9.60 ^a	0.2889
WBR	267.72±42.46 ^a	257.95±65.00 ^a	258.97±29.45 ^a	0.7819

^a Superscript letters indicate the absence of a statistical significance of $p > 0.05$.

With respect to the AC/TC ratio, the results demonstrate that the CONTR group had the lowest value (1.199) and that this value differed from that of the AMOX group ($p = 0.0329$). The AC/TC ratio indicates that a greater accumulation of abdominal fat occurred in the group that received amoxicillin alone [24, 26].

Table 3. BMI, Lee index, AC/TC ratio, TBW and body weight in the AMOX, AMOX + SB and CONTR groups

	AMOX	AMOX + SB	CONTR	p
BMI	0.599±0.053 ^a	0.606±0.072 ^a	0.544±0.055 ^b	0.0034
Lee index	0.287±0.011 ^a	0.286±0.013 ^a	0.274±0.011 ^b	0.0019
AC/TC ratio	1.254±0.090 ^a	1.250±0.054 ^b	1.199±0.066 ^b	0.0329
TBW, g	252.95±39.90 ^a	252.61±17.73 ^a	279.12±38.88 ^b	0.0243
Body weight, %	66.32±8.64 ^a	63.84±5.99 ^a	74.96±11.70 ^b	0.0012

Different superscript letters indicate a statistical significance of $p < 0.05$. Identical superscript letters indicate the absence of a statistical significance of $p > 0.05$.

TBW data demonstrate that although the CONTR group exhibited the lowest average weight gain (272.95 g) and the lowest final weight (375.10 g), this group had the greatest amount of body water (279.1 g, $p = 0.0243$). When evaluating the percentage of water in the bodies of the animals, this difference was even greater ($p = 0.0012$).

In the CONTR group, 74.96% of the body weight was water, while the AMOX and AMOX + SB groups exhibited body water percentages of 66.32 and 66.84%, respectively, with a p value of 0.0012. The body weight data demonstrate very clearly that a greater fat-free mass existed in the group that did not receive amoxicillin [27, 28].

Discussion

With respect to the weight gain of the animals, this study demonstrated that less weight gain occurred in the first 2 weeks for the group that received amoxicillin (fig. 1). This loss is explained by the diarrhea experienced during those 2 weeks by the animals that received the antibiotic (i.e. AMOX and AMOX + SB). From the third week on, there was an inversion, and the groups that received the antibiotic began to gain more weight. In week 8, this result became even more significant ($p = 0.0134$).

Although some authors reported similar results (i.e. changes in body composition occurred without weight changes [6, 29, 30]), the data from this study suggest that weight changes could appear in subsequent weeks if the study was extended. This prediction is plausible because in week 8 significant differences were observed between the groups that used amoxicillin and those that did not. Other studies found similar responses, where animals

and humans treated with antibiotics gained weight, particularly in cases in which the antibiotics were administered early in life [7, 31–33].

With respect to body composition and fat accumulation, the anthropometric data (i.e. BMI, Lee index and AC/TC ratio) and the TBIA data (i.e. TBW and BW) were consistent and demonstrated that the groups that were treated with amoxicillin exhibited a larger accumulation of body fat than the CONTR group.

The literature has reported this effect for years, since the first use of antibiotics as growth promoters in chickens, piglets and calves [34, 35]. This effect was discovered in the USA in 1946, when chickens were fed tetracycline derivatives [36].

Although some studies demonstrated the effectiveness of *S. boulardii* for resetting alterations caused by antibiotic treatment [37–39], we found no microbial protective effects in this study; the results of the group that received *S. boulardii* were identical to those of the group that received amoxicillin alone (i.e. an increase in body fat was observed).

The AMOX + SB group presented a higher weight gain (table 1) and a lower percentage of body water (table 3) than the group that received amoxicillin (AMOX) alone. Although this difference was not statistically significant, it may indicate a trend of weight gain promoted by the use of this probiotic.

This observation is endorsed by several studies that have shown that probiotics can promote gain or loss of weight in animals and humans, depending on the microorganism employed [40, 41]. In the particular case of *S. boulardii*, there are studies in children with diarrhea who had been treated with this probiotic and who gained more weight than the untreated group [42].

Other studies demonstrated the antiobesity effect of microorganisms such as *Bifidobacterium animalis* and the *Lactobacillus* species [40, 43], but this effect was not replicated with *S. boulardii* in the present study.

Recent studies of humans and animals have provided increasing evidence for a strong association between the consumption of antibiotics and weight gain and have demonstrated a significant role of the human microbiota in this process [6, 7, 44–48]. Studies of microbiota transplantation demonstrated that germ-free animals accumulated less fat, despite eating more. After receiving transplanted microbiota from obese animals, these animals gained more weight in fat [49, 50].

The role of the microbiota in obesity and the interference of antibiotics with the microbiota are well documented. More studies that manipulate the microbiota

with probiotics and prebiotics are needed to create a new front for the treatment of obesity.

On the other hand, studies using probiotics that promote weight gain, such as *S. boulardii*, should be encouraged to combat another major problem of the century, namely malnutrition.

Disclosure Statement

We wish to confirm that there are no known conflicts of interest associated with this publication and that there has been no significant financial support for this work that could have influenced its outcome.

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