Experimental Chemotherapy

Chemotherapy

Chemotherapy 2014;60:162–167
DOI: 10.1159/000371737

Received: August 15, 2014
Accepted after revision: December 22, 2014
Published online: March 18, 2015

Obesity and the Use of Antibiotics and Probiotics in Rats

Fernando de Sá Del Fiol Alessandra Cristina Marciano Tardelli Ferreira
Jorge José Marciano Maria Claudia Marques Luciane Lopes Sant'Ana

Department of Pharmacology, University of Sorocaba, Sorocaba, Brazil

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.

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

KARGER

E-Mail karger@karger.com
www.karger.com/che

0009–3157/15/0603–0162$39.50/0

Prof. Dr. Fernando de Sá Del Fiol
Universidade de Sorocaba, Rodovia Raposo Tavares
Sorocaba, SP 18023-000 (Brazil)
E-Mail fernando.fiol@prof.uniso.br

[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 (Presence®) 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-μA 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 nonconductive 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]: $TBW = 15.47 + 97.44\ BL^2/WBR$, 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.

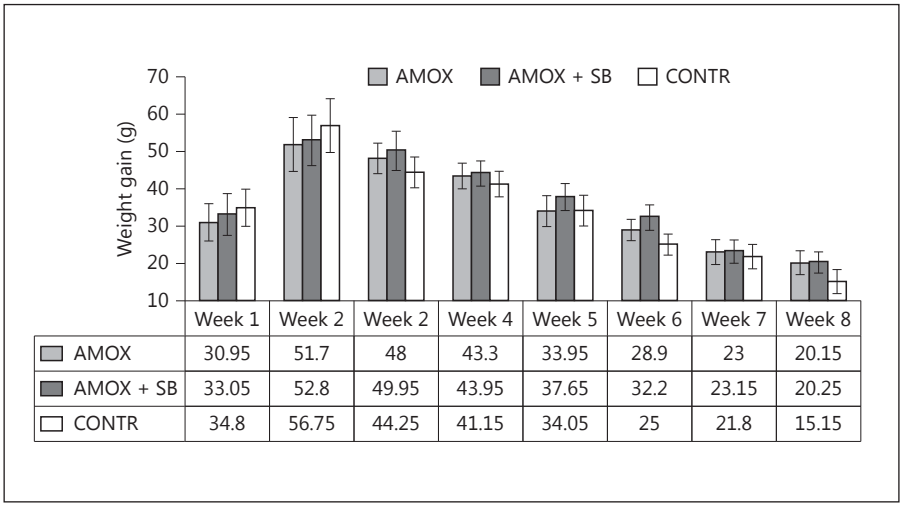

Fig. 1. Mean weekly weight gain in grams for the AMOX, AMOX + SB and CONTR groups.

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.

References

1 Murphy EF, Clarke SF, Marques TM, et al: Antimicrobials: strategies for targeting obesity and metabolic health? Gut Microbes 2013;4:48–53.
2 Riley LW, Raphael E, Faerstein E: Obesity in the United States – dysbiosis from exposure to low-dose antibiotics? Front Public Health 2013;1:69.
3 Newnham JP, Pennell CE, Lye SJ, Rampono J, Challis JR: Early life origins of obesity. Obstet Gynecol Clin North Am 2009;36:227–244, xii.
4 Million M, Lagier JC, Yahav D, Paul M: Gut bacterial microbiota and obesity. Clin Microbiol Infect 2013;19:305–313.
5 Maraki S, Mouzas IA, Kontoyiannis DP, Chatzinikolaou I, Tselentis Y, Samonis G: Prospective evaluation of the impact of amoxicillin, clarithromycin and their combination on human gastrointestinal colonization by *Candida* species. Chemotherapy 2001;47:215–218.
6 Cho I, Yamanishi S, Cox L, et al: Antibiotics in early life alter the murine colonic microbiome and adiposity. Nature 2012;488:621–626.
7 Trasande L, Blustein J, Liu M, Corwin E, Cox LM, Blaser MJ: Infant antibiotic exposures and early-life body mass. Int J Obes (Lond) 2013;37:16–23.
8 Ajslev TA, Andersen CS, Gamborg M, Sorensen TI, Jess T: Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. Int J Obes (Lond) 2011;35:522–529.
9 Libby DA, Schaible PJ: Observations on growth responses to antibiotics and arsonic acids in poultry feeds. Science 1955;121:733–734.
10 Gaskins HR, Collier CT, Anderson DB: Antibiotics as growth promotants: mode of action. Anim Biotechnol 2002;13:29–42.
11 Lin J: Antibiotic growth promoters enhance animal production by targeting intestinal bile salt hydrolase and its producers. Front Microbiol 2014;5:33.
12 Lin J, Hunkapiller AA, Layton AC, Chang YJ, Robbins KR: Response of intestinal microbiota to antibiotic growth promoters in chickens. Foodborne Pathog Dis 2013;10:331–337.
13 Reinhardt C, Reigstad CS, Backhed F: Intestinal microbiota during infancy and its implications for obesity. J Pediatr Gastroenterol Nutr 2009;48:249–256.
14 Agans R, Rigsbee L, Kenche H, Michail S, Khamis HJ, Paliy O: Distal gut microbiota of adolescent children is different from that of adults. FEMS Microbiol Ecol 2011;77:404–412.
15 Vael C, Verhulst SL, Nelen V, Goossens H, Desager KN: Intestinal microflora and body mass index during the first three years of life: an observational study. Gut Pathog 2011;3:8.
16 Markle JG, Frank DN, Mortin-Toth S, et al: Sex differences in the gut microbiome drive hormone-dependent regulation of autoimmunity. Science 2013;339:1084–1088.
17 Grzeskowiak L, Collado MC, Mangani C, et al: Distinct gut microbiota in southeastern African and northern European infants. J Pediatr Gastroenterol Nutr 2012;54:812–816.
18 Backhed F, Ley RE, Sonnenburg JL, Peterson DA, Gordon JI: Host-bacterial mutualism in the human intestine. Science 2005;307:1915–1920.
19 Salminen S, Isolauri E, Onnela T: Gut flora in normal and disordered states. Chemotherapy 1995;41(suppl 1):5–15.
20 Bibiloni MD, Pons A, Tur JA: Prevalence of overweight and obesity in adolescents: a systematic review. ISRN Obes 2013;2013:392747.
21 Ternak G: Antibiotics may act as growth/obesity promoters in humans as an inadvertent result of antibiotic pollution? Med Hypotheses 2005;64:14–16.
22 Hall CB, Lukaski HC, Marchello MJ: Estimation of rat body composition using tetrapolar bioelectrical impedance analysis. Nutr Rep Int 1989;39:627–633.
23 Yokoi K, Lukaski HC, Uthus EO, Nielsen FH: Use of bioimpedance spectroscopy to estimate body water distribution in rats fed high dietary sulfur amino acids. J Nutr 2001;131:1302–1308.
24 Novelli EL, Diniz YS, Galhardi CM, et al: Anthropometrical parameters and markers of obesity in rats. Lab Anim 2007;41:111–119.
25 Bernardis LL: Prediction of carcass fat, water and lean body mass from Lee's 'nutritive ratio' in rats with hypothalamic obesity. Experientia 1970;26:789–790.
26 Gerbaix M, Metz L, Ringot E, Courteix D: Visceral fat mass determination in rodent: validation of dual-energy X-ray absorptiometry and anthropometric techniques in fat and lean rats. Lipids Health Dis 2010;9:140.
27 Morbach CA, Brans YW: Determination of body composition in growing rats by total body electrical conductivity. J Pediatr Gastroenterol Nutr 1992;14:283–292.
28 Lesser GT, Deutsch S, Markofsky J: Fat-free mass, total body water, and intracellular water in the aged rat. Am J Physiol 1980;238:R82–R90.
29 Morel FB, Oosting A, Piloquet H, Oozeer R, Darmaun D, Michel C: Can antibiotic treatment in preweaning rats alter body composition in adulthood? Neonatology 2013;103:182–189.
30 Sheng HP, Huggins RA: A review of body composition studies with emphasis on total body water and fat. Am J Clin Nutr 1979;32:630–647.
31 Mozes S, Sefcikova Z, Bujnakova D, Racek L: Effect of antibiotic treatment on intestinal microbial and enzymatic development in postnatally overfed obese rats. Obesity (Silver Spring) 2013;21:1635–1642.
32 Southern KW, Barker PM, Solis-Moya A, Patel L: Macrolide antibiotics for cystic fibrosis. Cochrane Database Syst Rev 2012;11:CD002203.
33 Garly ML, Bale C, Martins CL, et al: Prophylactic antibiotics to prevent pneumonia and other complications after measles: community based randomised double blind placebo controlled trial in Guinea-Bissau. BMJ 2006;333:1245.
34 Dibner JJ, Richards JD: Antibiotic growth promoters in agriculture: history and mode of action. Poult Sci 2005;84:634–643.
35 Koluman A, Dikici A: Antimicrobial resistance of emerging foodborne pathogens: status quo and global trends. Crit Rev Microbiol 2013;39:57–69.
36 Moore PR, Evenson A, et al: Use of sulfasuxidine, streptothricin, and streptomycin in nutritional studies with the chick. J Biol Chem 1946;165:437–441.
37 Shan LS, Hou P, Wang ZJ, et al: Prevention and treatment of diarrhoea with *Saccharomyces boulardii* in children with acute lower respiratory tract infections. Benef Microbes 2013;4:329–334.
38 Szajewska H, Mrukowicz J: Meta-analysis: non-pathogenic yeast *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea. Aliment Pharmacol Ther 2005;22:365–372.

39 Kotowska M, Albrecht P, Szajewska H: *Saccharomyces boulardii* in the prevention of antibiotic-associated diarrhoea in children: a randomized double-blind placebo-controlled trial. Aliment Pharmacol Ther 2005;21:583–590.
40 Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D: Comparative meta-analysis of the effect of *Lactobacillus* species on weight gain in humans and animals. Microb Pathog 2012;53:100–108.
41 Angelakis E, Merhej V, Raoult D: Related actions of probiotics and antibiotics on gut microbiota and weight modification. Lancet Infect Dis 2013;13:889–899.
42 Le Luyer B, Makhoul G, Duhamel JF: A multicentric study of a lactose free formula supplemented with *Saccharomyces boulardii* in children with acute diarrhea (in French). Arch Pediatr 2010;17:459–465.
43 Yin YN, Yu QF, Fu N, Liu XW, Lu FG: Effects of four *Bifidobacteria* on obesity in high-fat diet induced rats. World J Gastroenterol 2010; 16:3394–3401.
44 Thuny F, Richet H, Casalta JP, Angelakis E, Habib G, Raoult D: Vancomycin treatment of infective endocarditis is linked with recently acquired obesity. PLoS One 2010; 5:e9074.
45 Pirzada OM, McGaw J, Taylor CJ, Everard ML: Improved lung function and body mass index associated with long-term use of macrolide antibiotics. J Cyst Fibros 2003;2:69–71.
46 Saiman L, Anstead M, Mayer-Hamblett N, et al: Effect of azithromycin on pulmonary function in patients with cystic fibrosis uninfected with *Pseudomonas aeruginosa*: a randomized controlled trial. JAMA 2010;303:1707–1715.
47 Ng YY, Su PH, Chen JY, et al: Efficacy of intermediate-dose oral erythromycin on very low birth weight infants with feeding intolerance. Pediatr Neonatol 2012;53:34–40.
48 Lane JA, Murray LJ, Harvey IM, Donovan JL, Nair P, Harvey RF: Randomised clinical trial: *Helicobacter pylori* eradication is associated with a significantly increased body mass index in a placebo-controlled study. Aliment Pharmacol Ther 2011;33:922–929.
49 Backhed F, Ding H, Wang T, et al: The gut microbiota as an environmental factor that regulates fat storage. Proc Natl Acad Sci USA 2004;101:15718–15723.
50 Turnbaugh PJ, Backhed F, Fulton L, Gordon JI: Diet-induced obesity is linked to marked but reversible alterations in the mouse distal gut microbiome. Cell Host Microbe 2008;3: 213–223.