# Interplay between weight loss and gut microbiota composition in overweight adolescents

Arlette Santacruz[1]; Ascensión Marcos[2], Julia Warnberg[2,3], Amelia Martí[4,5], Miguel Martin-Matillas[6], Cristina Campoy[6], Luis A. Moreno[7], Oscar Veiga[8], Carlos Redondo-Figuero[9], Jesús M. Garagorri[10], Cristina Azcona[5], Manuel Delgado[11], Miguel García-Fuentes[9], the EVASYON study group, Maria Carmen Collado[1] and Yolanda Sanz[1]*.

[1]Instituto de Agroquímica y Tecnología de Alimentos (CSIC), Apartado 73, 46100 Burjassot, Valencia, Spain; [2]Grupo de Inmunonutrición, Instituto del Frío (CSIC), Madrid, Spain.[3]Dept. Biosciences and Nutrition. Karolinska Institutet, Sweden. [4]Departamento de Medicina Preventiva y Salud Publica, Universidad de Navarra, Pamplona Spain; [5]Departamento de Fisiología y Nutrición, Universidad de Navarra, Pamplona Spain; [6]Departamento de Pediatría, Facultad de Medicina, Universidad de Granada, Granada, Spain; [7]EU Ciencias de la Salud, Universidad de Zaragoza, Spain; [8]Departamento de Educación Física, Deporte y Movimiento Humano, Universidad Autónoma de Madrid, Madrid, Spain. [9]Departamento de Ciencias Médicas y Quirúrgicas, Universidad de Cantabria, Santander, Spain; [10]Departamento de Pediatría, Radiología y Medicina Física, Universidad de Zaragoza, Spain. [11]Facultad de Ciencias de la Actividad Física y Deporte; Universidad de Granada, Spain.

***Corresponding author**: Yolanda Sanz, Instituto de Agroquímica y Tecnología de Alimentos (CSIC) PO Box 73, 46100 Burjassot, Valencia, Spain. Phone: +34 963900022; Fax: +34 963900022; E-mail: yolsanz@iata.csic.es

**Running head:** Weight loss and gut microbiota

**EVASYON Study Group** collaborators..

Coordinator: Marcos A. Local clinical treatment teams and researchers; (Principal Investigators are underlined) Campoy C, López-Belmonte G., Delgado M., Martín-Matillas M., Aparicio V., Carbonell A., Agil A., Silva D.R., Pérez-Ballesteros C., Piqueras M.J., Chillón P, Tercedor P, Martín-Lagos J.A., Martín-Bautista E., Pérez-Expósito M., Garófano M, Aguilar MJ, Fernández-Mayorga A, Sánchez P.; Madrid: Marcos A., Wärnberg J., Puertollano M.A., Gómez-Martínez S., Zapatera B., Nova E., Romeo J., Díaz E.L., Pozo T., Morandé G., Villaseñor A., Madruga D., Muñoz R, Veiga O., Villagra A., Martínez-Gómez D., Vaquero M.P., Pérez-Granados A.M, Navas-Carretero S.; Pamplona: Martí A., Azcona C., Moleres A., Rendo T. Marqués M., Miranda MG., Martínez J.A,; Santander: Redondo-Figuero C., García-Fuentes M., DeRufino P., González-Lamuño D., Amigo T., Sanz R.; Zaragoza: Garagorri J.M., Moreno L.A., Romero P., De Miguel-Etayo P., Rodríguez G., Bueno G., Mesana Mª. I., Vicente- Rodríguez G., Fernández J., Rey P., Muro C., Tomás C.; Data management and statistical analysis: Wärnberg J., Calle M.E., Barrios L.

**ABSTRACT**

The aim of this study was to determine the influence of an obesity treatment program on the gut microbiota and body weight of overweight adolescents. Thirty-six adolescents (13-15 years), classified as overweight according to the International Obesity Task Force body mass index (BMI) criteria, were submitted to a calorie-restricted diet (10-40%) and increased physical activity (15-23 kcal/kg body weight/wk) program over 10 weeks. Gut bacterial groups were analyzed by quantitative real-time PCR before and after the intervention. A group of subjects (n=23) experience more than 4.0 kg weight loss and showed significant BMI ($P$= 0.030) and BMI z-score ($P$= 0.035) reductions after the intervention, while the other group (n=13) showed less than 2.0 kg weight loss. No significant differences in dietary intake were found between both groups. In the whole adolescent population, the intervention led to increased *Bacteroides fragilis* group ($P$=0.001) and *Lactobacillus* group ($P$=0.030) counts, and to decreased *C. coccoides* group ($P$=0.028), *B. longum* ($P$=0.031) and *B. adolescentis* ($P$=0.044) counts. In the high weight-loss group, *Bacteroides fragilis* group and *Lactobacillus* group counts also increased ($P$=0.001 and $P$=0.007, respectively), whereas *Clostridium coccoides* group and *B. longum* counts decreased ($P$=0.001 and $P$=0.044, respectively) after the intervention. Total bacteria, *Bacteroides fragilis* group, *C. leptum* group and *B. catenulatum* groups counts were significantly higher ($P$<0.001-0.036) while levels of *C. coccoides* group, *Lactobacillus* group, *Bifidobacterium*, *B. breve* and *B. bifidum* were significantly lower ($P$<0.001-0.008) in the high weight-loss group than in the low weight-loss group before and after the intervention. These findings indicate that calorie restriction and physical activity have an impact on gut microbiota composition related to body weight loss, which also seem to be influenced by the individual's microbiota.

## INTRODUCTION

Obesity is viewed as one of the major current public-health problems and its impact is highest in children, contributing to significant morbidity in adulthood (1). The development of metabolic complications, associated with obesity during childhood, have repercussions in adulthood, increasing the risk of type-2 diabetes and premature cardiovascular diseases (2). A link is thought to exist between obesity, chronic low-grade inflammation, insulin resistance and endothelial dysfunction (3, 4). The risk factors for childhood obesity include diet, low socioeconomic status, parental obesity, rapid infancy weight gain, and decreased physical activity (5). Obesity prevention programs based on changes in school and community environments can decrease childhood weight gain to a limited extent (5). Therefore, further studies on dietary and host factors with an impact on energy balance are needed to improve the intervention strategies and measures for obesity control over time.

Recent reports have suggested that gut microbiota is an important factor affecting energy disposal and storage in adipocytes (6, 7). The gut microbiota is also known to be involved in modulation of host immunity, and the inflammatory status associated with obesity in mice (8, 9). However, the precise mechanisms by which alterations in microbiota affect obesity and associated disorders are still unclear.

It has been reported how diets based on a high protein intake and/or low carbohydrate intake, or high fat intake may alter microbial composition and activity in the large intestine and thus exert an impact on gut health (6, 8-10). Nevertheless, knowledge of the interactions between energy intake and specific microbial populations, and their influence on body weight, are limited to small-scale clinical trials (7). Specific studies in obese adolescents, who represent a high-risk population group, are lacking.

The objective of this work was to determine the influence of a multidisciplinary obesity treatment program, comprising a calorie-restricted diet and physical activity, on the structure of the fecal microbiota of overweight and obese adolescents and its relation to dietary intake and weight loss by analyzing the main gut bacterial groups and *Bifidobacterium* species by quantitative real-time PCR.

## RESEARCH METHODS AND PROCEDURES

### Subjects and anthropometric measures

Subjects for the study were selected according to their body mass index (BMI) [weight (kg)/[height (m)$^2$]. Childhood overweight (including obesity) was defined according to the International Obesity Task Force cut-offs for BMI (11). BMI z-scores were calculated as a function of the subject's obesity degree when compared with BMI local reference standards (12). Body weight (kg) was estimated without shoes and with light clothing, and measured to 0.05 kg by using a standard beam balance. Skinfold thickness was measured on at the left side of the body to the nearest 0.1 mm using a Holtain skinfold caliper at triceps, biceps, subcapular, suprailiac, thigh, and calf, as previously described (12). All the anthropometric variables were measured in order, three times and averaged. For all the anthropometric measurements, intra-observer reliability was higher than 95% and inter-observer reliability was higher than 90%. The characteristics of the thirty-six selected adolescents (18 female and 18 male; mean age: 14.5 years) to be submitted to the obesity-treatment program are shown in Table 2. None of the volunteers were treated with antibiotics for at least 1 month before the intervention study and during the study. The study was conducted in accordance with the ethical rules of the Helsinki Declaration (Hong Kong revision, September 1989), following the EEC Good Clinical Practice guidelines (document 111/3976/88 of July 1990) and current Spanish law which regulates clinical research in humans (Royal Decree

561/1993 regarding clinical trials). Informed consent was obtained from all adolescents and their parents, and the study was approved by the local Ethics Committees**.**

**Intervention**

Over a 10-week period, the participants were subjected to the intervention based on an energy-restricted diet (a 10-40% reduction) established according to both obesity degree and regular physical activity (13). The maximum energy intake was 1800 kcal/day for females and 2200 kcal/day for males. The physical activity was determined by accelerometry and exercise prescribe at least 1 hour of moderate to vigorous intensity 3 or 5 days per week, depending of the individual physical activity level. The energy expenditure was estimated in MET´s values (14) for each activity and the frequency and intensity of the activities of the exercise program (walking, biking, running, swimming, etc.). The energy expenditure range obtained was from 15 to 23 Kcal/Kg of body weight per week. Diet energy content was set from the resting energy expenditure calculated with the Schofield equation multiplied by 1.3 as physical activity factor (13). Energy restriction was calculated in function of the subject obesity degree: 10% restriction when the subject had a BMI between 0 to 2 standard deviations (SD) above the mean, 20% with BMI between 2 to 3 SD above the mean, 30% between 3 to 4 SD and 40% if the subject had a BMI >4 SD above the mean according to BMI local reference standards. Macronutrient distribution was 50% of energy from carbohydrates, 30 % from fat and 20 % from proteins. Energy distribution during the day was: breakfast: 20 % of daily energy; morning snack: 10-15 % of daily energy; lunch: 30-35 % of daily energy; afternoon snack: 5-10 % of daily energy; dinner: 20-25 % of daily energy.

**Dietary assessment**

Food diary records were kept for 72h (2 weekdays and 1 weekend day) both before the start of the study (baseline intakes) and after the intervention (week 10). Detailed information on how to record food and drink consumed using common household measures was provided. Food diary records were returned to their dietician, and analyzed for energy, water and nutrient contents based on the CESNID food-composition database of Spanish foods (15). Starches were defined as complex carbohydrates and fiber was computed as total non-digestible carbohydrates (soluble and non-soluble). Due to limitations of the food composition database (15) and also the inherent limitation of dietary assessment in free living young populations, no further details are available according to other key nutrients that are proved to serve as substrate for the gut microbiota (i.e. resistant starch, oligosaccharides or fructans).

**Fecal and DNA sample preparation**

Fecal samples were kept immediately after collection at -20 ºC and stored until analyzed. Samples were diluted 1: 10 (w/v) in PBS (pH 7.2), homogenized and one aliquot was used for DNA extraction by using the QIAamp DNA stool Mini kit (Qiagen, Hilden, Germany).

**Microbial analysis by quantitative real-time PCR (qPCR)**

Specific primers targeting different bacterial genera and species were used to characterize the fecal microbiota by qPCR (Table 1), essentially as described previously (16-20). Briefly, PCR amplification and detection were performed with an ABI PRISM 7000-PCR sequence detection system (Applied Biosystems, UK). Each reaction mixture of 25 μL was composed of SYBR® Green PCR Master Mix (SuperArray Bioscience Corporation, USA), 1 μL of each of the specific primers at a concentration of 0.25 μM, and 1 μL of template DNA. Bacterial concentration from each sample was calculated by

comparing the Ct values obtained from the standard curves. Standard curves were created using serial 10-fold dilution of pure cultures of DNA, corresponding to $10^2$ to $10^9$ cells from the culture collection as determined by microscopy counts using DAPI. The following strains were used as references: *Bacteroides fragilis* DSMZ 2451, *Clostridium coccoides* DSMZ 933, *C. leptum* DSMZ 935, *Lactobacillus casei* ATCC 393, *E. coli* CECT 45, *Bifidobacterium longum* subsp. *longum* CECT 4503, ***B. bifidum*** LMG 11041, *B. breve* LMG 11042, *B. pseudocatenulatum* CECT 5776, *B. adolescentis* LMG 11037. The strains were obtained from the Spanish Collection of Type Cultures (CECT) and the German Collection of Microorganisms and Cell Cultures (DSMZ).

**Statistical analyses**

Statistical analyses were done using the SPSS 11.0 software (SPSS Inc, Chicago, IL, USA). Due to non-normal distribution, microbial data are expressed as medians with interquartile ranges (IQR) and differences in bacterial populations were determined by applying the Mann–Whitney *U* test and the Wilcoxon Signed Rank Test. Correlations among variables were calculated by using the Spearman's correlation test. Differences in clinical and anthropometric data were also determined by applying the Mann–Whitney *U* test. Dietary composition (means and standard deviations) were calculated for crude (unadjusted) nutrients from the 72 h dietary registers and data were averaged for the analysis. All dietary variables submitted to log-transformation showed fit normal distribution. Repeated-measures ANOVA analysis adjusted for sex and age was used to examine differences in group mean intake before (baseline) versus after the intervention. In every case, *P*-values <0.050 were considered statistically significant.

## RESULTS

**Subjects and obesity intervention program**

The studied subjects, 50 % female (18/36) and 50 % male (18/36), were 14.5 years old (13.0-15.0 y) and maintained an apparently good health status during the study. Clinic and anthropometric characteristics did not differ significantly among subjects at recruitment time, particularly regarding weight (*P*= 0.266), BMI (*P*= 0.221), and BMI-z score (P= 0.138) and, therefore, they were comparable. (Table 2). The subjects showed marked differences in weight loss after intervention and, accordingly, subdivided into two groups as low weight-loss group (<2.0 kg of weight loss, n=13) and high weight-loss group (>4.0 kg of weight loss after intervention, n=23). The median of weight loss after 10 weeks under the intervention program for the first group was of 1.4 (0.75-2.00) Kg, corresponding to 1.3 % (IQR 0.85-2.25 %) of body weight. This group did not showed significant differences in BMI (*P*= 0.545), weight (*P*= 0.801) and BMI z-score (*P*= 0.579) before and after the dietary intervention. In the second group, the median of weight loss after 10 weeks under the intervention program was of 6.8 (4.8-9.0) Kg, corresponding to 7.5 % (IQR 5.8-9.3 %) of body weight, without detecting significant differences between male (*P*= 0.204) and female (*P*= 0.083). In this group significant differences in BMI (*P*= 0.030) and BMI z-score (*P*= 0.035) were detected before and after the intervention.

Dietary data before and after the intervention of the low weight and high weight-loss groups are shown in Table 3. No interaction between time (before and after intervention) per sex or age-group was observed. No significant differences in dietary intake of energy, macronutrients or on food group level were found between groups before and after the intervention program. The consumption of probiotics *i.e* yogurt was almost one portion per day (0.9 portions in both groups, one portion in Spain is equivalent to 125g). None of the subjects consumed pre- or probiotics as supplements. The main sources of carbohydrates, in order of increasing intakes per day, were cereals,

potatoes, fruits and diary products. The main fiber sources of this population were vegetables, cereals, fruits and legumes.

In both adolescent groups, the dietary intervention mainly resulted in a significant reduction ($P< 0.05$) in intake of total energy (63.8 % mean reduction; SD 1.2) and macronutrients including proteins (74.5 % mean reduction, SD 27.2), fat (51.8 % mean reduction; SD 3.8), polyunsaturated fatty acids (PUFA) (48.7% mean reduction, SD 12.5), carbohydrates (71.6% mean reduction, SD 3.9), simple carbohydrates (73.3% mean reduction; SD 0.8), and complex carbohydrates (70.6% mean reduction; SD 7.2). The reduction in complex carbohydrate intake was significantly and negatively correlated (R=-0.334; $P= 0.050$) to changes in *Bacteroides fragilis g*roup as a result of the intervention. Likewise, reduction in PUFA intake was almost significantly and negatively correlated (R=-0.313, $P=0.063$) to changes in *Lactobacillus* group counts.

**Influence of intervention in fecal bacterial group composition**

Inter-individual differences on fecal microbiota composition for all studied adolescents were 0.77 (IQR 0.39-1.70) for *Bacteroides fragilis* group, -0.36 (IQR -0.82-0.29) for *Bifidobacterium*, -0.65 (IQR -0.98—0.27) for *C. coccoides* group, 0.02 (IQR -0.50-0.45) for *C. leptum* group, 0.10 (IQR -0.38-0.49) for *E. coli* and 0.43 (IQR 0.09-0.83) for *Lactobacillus* group.

The intervention in whole adolescent population (n=36) resulted in increased counts of *Bacteroides fragilis* group ($P=0.001$) and *Lactobacillus* group ($P=0.030$), and decreased counts of *C. coccoides* group ($P=0.028$). No significant differences were found in the other bacterial groups analyzed. *Bacteroides fragilis* group (R= 0.55, $P$-value < 0.001), and *C. leptum* group (R=0.52, $P$-value< 0.001) counts after the intervention significantly correlated with higher weight loss (kg), while the opposite correlations were found for the *E. coli* (R=-0.26, $P$-value=0.025), *C. coccoides* group (R=-0.61, $P$-

value < 0.001), *Lactobacillus* group (R=-0.40, *P*-value=0.001) and *Bifidobacterium* (R=-0.37, *P*-value=0.001) counts.

Changes in bacterial counts as a result of the intervention were also evaluated by considering separately the high and the low weight-loss groups (Tables 4 and 5). Significant differences were not found in bacterial counts of any of the analyzed groups before and after intervention in the low weight-loss group (n=13 and <2.0 kg of weight loss; Table 4), while significant differences were found in the high weight-loss group (n= 23 and >4.0 kg of weight loss; Table 5). In this last group, *Bacteroides fragilis* group and *Lactobacillus* group counts significantly increased (*P*=0.001 and *P*=0.007, respectively), while those of the *C. coccoides* group significantly decreased (*P*=0.001) after 10 weeks of intervention. Moreover, the ratio of *Bifidobacterium* to *C. coccoides* group counts increased significantly after the intervention (*P*=0.022) when compared to the ratio recorded beforehand, while the ratio of *Bifidobacterium* to *Bacteroides fragilis* group counts decreased (*P*=0.001). When subjects of high weight-loss group were classified according to gender, certain significant differences were found between the two groups. In females, *Bacteroides fragilis* group significantly increased (*P*=0.002) after the intervention, while *C. coccoides* group counts decreased (*P*=0.023), which was in accordance with the results obtained when considering the total high weight-loss group of adolescents. *Lactobacillus* group increased but the differences were not statistically significant. In males, *Lactobacillus* and *Bacteroides fragilis* groups increased significantly (*P*=0.001 and *P*= 0.033, respectively) after the intervention, whereas a significant (*P*=0.007) reduction was found in the *C. coccoides* group, as was detected for the total high weight-loss group of adolescents.

Significant correlations between bacterial counts after the intervention and weight loss were found in the high weight-loss group (Figure 1). Increased levels of *Bacteroides fragilis* group (R= 0.27, *P*-value=0.055) and *Lactobacillus* group significantly

correlated (R=0.55, *P*-value<0.001) with weight loss (kg), while the opposite correlation (R=-0.37, *P*-value=0.010) was found for the *E. coli* (Figure 1). Similar correlations were recorded between *Lactobacilllus* group (R= 0.53, *P*-value=0.008) and *Bacteroides fragilis* group (R=0. 44, *P*-value=0.036) levels, and body weight-loss percentages. The reductions in BMI z-scores as a result of the intervention were also significantly correlated with increased levels of *Lactobacillus* group (R= 0.64, *P*-value= 0.001) and *Bacteroides fragilis* group (R= 0.46, *P*-value= 0.025). Reduced *Clostridium coccoides* group levels were related to weight loss (R=- 0.611, *P*=0.001). The correlation between the reduction in *Bifidobacterium* to *C. coccoides* group ratio and weight loss was significantly (R=0.25, *P*- value=0.030), as well as the correlation between the reduction in *Bifidobacterium* to *Bacteroides fragilis* group ratio and weight loss (R=-0.62, *P*- value<0.001) as a result of the intervention.

**Influence of intervention in *Bifidobacterium* species composition**

In the whole adolescent population (n=36), total *Bifidobacterium* group counts were similar before and after intervention, while *B. longum* and *B. adolescentis* counts were significantly lower after intervention than before (*P*=0.031 and *P*=0.044, respectively). No significant differences were found in the other *Bifidobacterium* species analyzed. *B. breve* (R= -0.56, *P*-value < 0.001), and *B. bifidum* (R=-0.76, *P*-value< 0.001) counts after the intervention significantly correlated with lower weight loss (kg), while no correlations were found in the other species.

Changes in *Bifidobacterium* species counts as a result of the intervention were also evaluated by considering separately the high and the low weight-loss groups (Table 4 and 5). *Bifidobacterium* species counts showed significant differences as a result of the intervention in the high weight-loss group (Table 5), while not in the low weight-loss group of adolescents (Table 4). In the high weight-loss group, all *Bifidobacterium*

species analyzed decreased after the dietary intervention, although only the changes in *B. longum* counts were significant ($P$=0.044). Similar trends were found when comparing *Bifidobacterium* species composition in males or females. However, only *B. adolescentis* counts decreased significantly after intervention ($P$=0.037) in males, whereas no significant differences were found in females. Significant correlations were not detected between *Bifidobacterium* species counts and either weight loss, BMI or BMI z-score.

**Differences in fecal microbiota composition between the low weight-loss and high weight-loss groups of adolescents**

The differences in fecal microbiota composition between low- and high weight-loss groups of adolescents before and after the intervention are shown in Table 6. Before the intervention, total bacteria, *Bacteroides fragilis* group and *C. leptum* group counts were significantly higher ($P$<0.001, $P$=0.004 and $P$<0.001, respectively), while those of *C. coccoides* group, *Lactobacillus* group and *Bifidobacterium* were significantly lower ($P$<0.001, $P$<0.001 and $P$=0.001, respectively) in the high weight-loss group than in the low weight-loss group. The ratio of *Bacteroides fragilis* group to *C. coccoides* group was also significantly higher ($P$< 0.001) in the high weight-loss group. The same trend was detected for *Bifidobacterium* to *C. coccoides* group ratio but the differences were not significant ($P$= 0.140). After 10 weeks of intervention, similar differences on microbiota were found between the low weight and the high weight-loss groups. Total bacteria, *Bacteroides fragilis* group and *C. leptum* group counts were significantly higher ($P$=0.015, $P$=0.001 and $P$<0.001, respectively), while counts of the *C. coccoides* group, *Lactobacillus* group and *Bifidobacterium* were significantly lower ($P$<0.001, $P$<0.001 and $P$=0.008, respectively) in the high weight-loss than in the low weight-loss group. In addition, *Bacteroides fragilis* group, *Bifidobacterium* and *Lactobacillus* group

to *C. coccoides* group ratios were significantly higher ($P<0.001$, $P<0.001$ and $P=0.034$, respectively) in the high weight-loss than in the low weight-loss group.

In relation to *Bifidobacterium* species composition, *B. breve* and *B. bifidum* group counts were significantly higher in the low weight-loss group than in the high weight-loss group before ($P=0.001$ and $P< 0.001$, respectively) and after intervention ($P<0.001$ for both groups), whereas *B. catenulatum* group levels were higher in high weight-loss group ($P=0.030$ and 0.036, before and after intervention, respectively).

## DISCUSSION

This study shows for the first time that an intervention based on both a reduction in energy intake and an increase in energy expenditure has an important impact on the composition of the gut microbiota of overweight adolescents related to body weight loss. *Bacteroides fragilis* group and *Lactobacillus* group seem to be the gut bacteria most amenable to dietary intervention on the basis of the relationships established between the shifts of these bacterial counts and complex carbohydrate and PUFA intakes during the intervention. The *Bacteroides* genus has been shown to have high ability to utilized complex carbohydrates, which may explain the aforementioned correlation (21). A possible correlation between PUFA intake and *Lactobacillus* group count reductions was also detected, suggesting that PUFA intake may favor the prevalence of *Lactobacillus* group in the gut microbiota. In previous studies, PUFA have been shown to be utilized by *Lactobacillus*, leading to changes in bacterial fatty acids and suggesting a potential role of *Lactobacillus* as regulators of PUFA absorption *in vivo* (22). In addition, PUFA have positively influenced the adhesion of *Lactobacillus* to the jejunal mucosa of gnotobiotic piglets, indicating that the intake of these fatty acids may influence the intestinal levels of this bacterial group (23). Nevertheless, the extent to which these bacterial group counts may change and influence weight loss do

not seem to depend only on the diet since significant differences in bacterial counts but not in dietary intakes were detected between the high weight-loss and the low-weight loss groups during the intervention. Thus, these findings suggest that the individual's gut microbiota is an additional factor contributing together with lifestyle to body weight regulation.

In response to the intervention, levels of the *Bacteroides fragilis* group significantly increased and correlated to weight loss and BMI z-score reductions, while those of the *C. coccoides* group, which comprises the *Clostridium* cluster XIVa including members of other genera such as *Coprococcus*, *Eubacterium*, *Lachnospira*, and *Ruminococcus* (17), decreased and correlated to weight loss in the whole adolescent population and in the high weight-loss group. These findings were in agreement with the results previously obtained in the same population by using fluorescent *in situ* hybridization (FISH) technique, which showed that proportions of *C. histolyticum,* and *E. rectale-C. coccoides* groups dropped and those of the *Bacteroides-Prevotella* group increased after the intervention in those adolescents that lost more than 4 kg (24). In other studies, the fecal microbiota of obese adult subjects also showed a significant increase in *Bacteroidetes* and a proportional decrease in *Firmicutes* (which included *Clostridium* genus) after following either a fat- or carbohydrate-restricted low-calorie diet, which led to weight loss over a year (7). Thus, the association between *Bacteroides fragilis* group and *C. coccoides* group with energy intake and body weight changes confirmed in this short-term intervention study by using different molecular detection techniques resembles that previously established with the broad phyla *Bacteroidetes* and *Firmicutes* in a human long-term intervention study (7).

In this study, the ratio of *Bifidobacterium* to *Clostridium coccoides* group counts significantly increased as a result of the intervention in the high weight-loss group. A significant reduction of this ratio was also evident in children who developed atopic

diseases later, indicating that the relative proportions of these bacterial groups may precede the development of immune-related disorders (25). Thus, a reduction in calorie intake and an increase in energy expenditure may also have a beneficial overall impact on these bacterial populations and their relationship to the pro-inflammatory status linked to obesity. However, the intervention led to reductions in *B. longum* and *B. adolescentis* counts in the whole adolescent population as well as to reductions in *B. longum* and *B. adolescentis* counts in the high weight-loss group and in males of this group, respectively. A reduced dietary intake of carbohydrates by obese adult subjects was shown to be associated with reductions in *Bifidobacterium* counts in previous studies (10), which could also partly explained the reductions of this bacterial groups in the studied adolescents. In fact, genomic and physiological studies have shown that species such as *B. longum* and *B. adolescentis* may actively participate in the utilization of complex polysaccharides in the colon (21). In general, beneficial effects have previously been attributed to *Bifidobacterium* in connection with obesity. In obese mice models fed with a high fat-content diet, increases in *Bifidobacterium* caused by administering a high fermentable oligosaccharide were positively correlated with the normalization of inflammatory status, improved glucose tolerance and glucose-induced insulin secretion (8, 9). In addition, reductions in *Bifidobacterium* populations have been shown to precede the development of overweight (26). It is likely that relative proportions of *Bifidobacterium* to other bacterial groups, like those detected in this study in relation to *Clostridium*, rather than absolute numbers have a meaning in the context of obesity.

In general, although some of the reported differences in bacterial counts associated to body weight loss were small, from the biological point of view, these differences could be important in the long-term by themselves and because they may lead to changes in

the relative proportions of other intestinal bacteria competing for the same ecological niche, which may exert a mild but sustained effect on energy metabolism.

Interestingly, significant increases in *Lactobacillus* group counts in the whole adolescent population and in the high weight-loss group were detected after the intervention, in agreement with the trend previously detected by FISH analyses although the differences were not significant (24). In this study, the increase in *Lactobacillus* group counts was correlated with weight loss and BMI z-score reductions in the high weight-loss group, pointing to a role for this bacterial group in body-weight management. Until now, information about the impact of different diets on *Lactobacillus* group levels was scarce. In a recent human study *Lactobacillus* group levels were not significantly modified after following different diets: high-protein and low-carbohydrate diet or a high-protein and moderate-carbohydrate diet (10). In mice fed with a high fat-content diet no significant differences were found in *Lactobacillus* group levels as compared to controls (8, 9).

The gut microbiota of adolescents also appeared to be different between subjects showing high weight-loss and low weight-loss during the intervention and, apparently, this feature was not related to significant differences in dietary intakes. The adolescent group, which showed higher counts of total bacteria, *Bacteroides fragilis* group, *C. leptum* group and *B. catenulatum* group, and lower counts of *C. coccoides* group, *Lactobacillus* group*, Bifidobacterium, B. breve* and *B. bifidum* in their fecal microbiota, was the one that experienced the highest weight loss under the intervention. In addition, *Bacteroides fragilis* group*, Bifidobacterium* and *Lactobacillus* group to *C. coccoides* group ratios were higher in the high weight-loss group than in the low weight-loss group. Thus, *Bacteroides fragilis* and *C. coccoides* group counts of the individual's microbiota seemed to oppositely influence the ability of the host to loss weight under the same dietary intervention in agreement with the detected correlations between these

bacterial groups and weight loss. The opposite influences that seem to exert these bacterial groups on body weight are in agreement with previous reports in obese mice models and in a small-scale trial with adult human subjects (6-7). In this context, although increased counts of *C. leptum* group, which includes certain members of the genera *Clostridium*, *Ruminococcus*, *Eubacterium*, and *Faecalibacterium* that belong to *Clostridium* cluster IV (17), also seemed to favor weight loss, this trend was not confirmed when comparing the bacterial counts of this group before and after the intervention in the high weight-loss group. In addition, reduced *B. bifidum* and *B. breve* counts and increased *B. catenulatum* counts seemed to favor weigh loss, but these trends were not confirmed by the changes detected before and after the intervention in the high weight-loss group. Therefore, further studies are needed to draw conclusions about the role of specific *Bifidobacterium* species in obesity and weight management. In addition, the possibility that the low weight-loss group did not respond to the intervention due to failure to comply completely with the diet cannot be completely disregarded, since it is well recognized that obese patients have difficulty to accurately record their own food intake.

In summary, an association of specific bacterial groups with obesity and body weight loss has been reported in adolescents, pointing to a role played by *Bacteroides fragilis, Lactobacillus* and *Clostridium coccoides* groups, as well as by the relative proportions of *Bacteroides, Bifidobacterium* and *Lactobacillus* to *C. coccoides*. The obtained results have also indicated that the interactions between the gut microbiota and body weight may be sensitive to lifestyle intervention to different extent depending on the individual's microbiota structure.

**ACKNOWLEDGEMENTS**

This work was supported by grants AGL2007-66126-C03-01/ALI and Consolider Fun-*C*-Food CSD2007-00063 from the Spanish Ministry of Science and Innovation and AP 002/07 from Consejería de Sanidad (Valencia, Spain). The EVASYON study was supported by grants from Spanish Ministry of Health (PI052451, PI050855, PI051080, PI052369, PI051579). The scholarship from CONACYT (México) to A. Santacruz, and I3P Postdoctoral Contract from CSIC (Spain) to MC Collado are fully acknowledged. The collaborators in the EVASYON study Group are also acknowledged and especially the dietitians P Romero, P de Miguel, T Rendo and MJ Piqueras for the dietary analysis included in this paper,

**DISCLOSURE**

The authors declared no conflict of interest.

## References


1. Owen C, Martin R, Whincup P, Smith D, Cook D. The effect of infant feeding on the risk of obesity across the life course: a quantitative review of published evidence. *Pediatrics* 2006; 115: 1367-1377.
2. Nathan BM, Moran A. Metabolic complications of obesity in childhood and adolescence: more than just diabetes. *Curr Opin Endocrinol Diabetes Obes* 2008; 15:21-29.
3. Wärnberg J, Nova E, Moreno LA, *et al*. Inflammatory proteins are related with total and abdominal adiposity in a healthy adolescent population. The AVENA study. *Am J Clin Nutr* 2006; 84:505-512.
4. Hotamisligil GS. Inflammation and metabolic disorders. *Nature* 2006; 14: 444:860-867.
5. Isganaitis E, Levitsky LL. Preventing childhood obesity: can we do it?. *Curr Opin Endocrinol Diabetes Obes* 2008; 15:1-8.
6. Bäckhed F, Ding H, Wang T, *et al*. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci* 2004; 101:15718-15723.
7. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature* 2006; 444:1022-1023.
8. Cani PD, Amar J, Iglesias MA, *et al*. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; 56:1761–1772.
9. Cani PD, Neyrinck AM, Fava F, *et al*. Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. *Diabetologia* 2007; 50:2374-2383.
10. Duncan SH, Belenguer A, Holtrop G, Johnstone AM, Flint HJ, Lobley E. Reduced dietary intake of carbohydrates by obese subjects results in decreased concentrations

of butyrate and butyrate-producing bacteria in feces. *Appl Environ Microbiol* 2007; 73:1073-1078.

11. Cole TJ, Bellizzi MC, Flegal KM, Dietz WH. Establishing a standard definition for child overweight and obesity worldwide: international survey. *BMJ* 2000; 320:1-6.
12. Moreno LA, Mesana MI, González-Gross M, *et al*. Anthropometric body fat composition reference values in Spanish adolescents. The AVENA Study. *Eur J Clin Nutr* 2006; 60: 191–196.
13. Rodriguez G, Moreno LA, Sarría A, Fleta J, Bueno M. Resting energy expenditure in children and adolescents: agreement between calorimetry and prediction equations. *Clin Nutr* 2002; 21:255-260.
14. Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR Jr, Schmitz KH, Emplaincourt PO, Jacobs DR Jr, Leon AS. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000 Sep;32(9 Suppl):S498-504.
15. Farran A, Zamora R, Cervera P, CESNID. *Tablas de composición de alimentos CESNID*. 2ª ed. Edicions Universitat de Barcelona - McGraw-Hill /Interamericana: Barcelona, 2004.
16. Matsuki T, Watanabe K, Fujimoto J, *et al*. Development of 16S rRNA-gene-targeted group-specific primers for the detection and identification of predominant bacteria in human feces. *Appl Environ Microbiol* 2002; 68: 5445-5451.
17. Matsuki T, Watanabe K, Fujimoto J, Takada T, Tanaka R. Use of 16S rRNA gene-targeted group-specific primers for real-time PCR analysis of predominant bacteria in human feces. *Appl Environ Microbiol* 2004;70:7220-7228.
18. Malinen E, Rinttilä T, Kajander K, *et al*. Analysis of the fecal microbiota of irritable bowel syndrome patients and healthy controls with real-time PCR. *Am J Gastroenterol* 2005; 100:373-382.

19. Walter J, Hertel C, Tannock GW, Lis CM, Munro K, Hammes WP. Detection of *Lactobacillus*, *Pediococcus*, *Leuconostoc*, and *Weissella* species in human feces by using group-specific PCR primers and denaturing gradient gel electrophoresis. *Appl Environ Microbiol* 2001; 67:2578-2585.

20. Heilig HG, Zoetendal EG, Vaughan EE, Marteau P, Akkermans ADL, de Vos WM. Molecular diversity of *Lactobacillus* spp. and other lactic acid bacteria in the human intestine as determined by specific amplification of 16S ribosomal DNA. *Appl Environ Microbiol* 2002; 68:114-123.

21. Sanz Y, Santacruz A, De Palma G. Insights into the roles of gut microbes in obesity. The Human Microbiome and Infectious Diseases: Beyond Koch. *Interdisciplinary Perspectives on Infectious Diseases*. 2008; doi: 10.1155/2008/829101.

22. Kankaanpää P, Yang B, Kallio H, Isolauri E, Salminen S. Effects of polyunsaturated fatty acids in growth medium on lipid composition and on physicochemical surface properties of lactobacilli. *Appl Environ Microbiol*. 2004;70:129-136.

23. Bomba A, Nemcová R, Gancarcíková S, Herich R, Pistl J, Révajová V, Jonecová Z, Bugarský A, Levkut M, Kasteĺ R, Baran M, Lazar G, Hluchý M, Marsálková S, Posivák J. The influence of omega-3 polyunsaturated fatty acids (omega-3 pufa) on lactobacilli adhesion to the intestinal mucosa and on immunity in gnotobiotic piglets. *Berl Munch Tierarztl Wochenschr*. 2003;116:312-316.

24. Nadal I, Santacruz A, Marcos A, Warnberg J, Garagorri M, Moreno LA, Martin-Matillas M, Campoy C, Martí A, Moleres A, Delgado M, Veiga OL, García-Fuentes M, Redondo CG, Sanz Y. Shifts in clostridia, bacteroides and immunoglobulin-coating fecal bacteria associated with weight loss in obese adolescents. *Int J Obes (Lond)* 2008; 9:1-10.

25. Kalliomäki M, Kirjavainen P, Eerola E, Kero P, Salminen S, Isolauri E. Distinct patterns of neonatal gut microflora in infants in whom atopy was and was not developing. *J Allergy Clin Immunol* 2001; 107:129-134.

26. Kalliomäki M, Collado MC, Salminen S, Isolauri E. Early differences in faecal microbiota composition in children may predict later weight-gain? *Am J Clin Nutr* 2008; 87: 534-538.

**List of Tables**



**List of figures**



**Figure Legend**

**Figure 1.** Correlations between fecal bacterial counts and weight loss after intervention in the high-weight loss group (n=23; >4.0 kg weight loss) of adolescents. Lines showed the Pearson correlation (linear adjustment). A) *Lactobacillus* group vs. weight loss; B) *E. coli* vs. weight loss; C) *Bacteroides fragilis* group vs. weight loss.

**Table 1. Oligonucleotide primers used in this study.**

| Target bacterial group/species | Sequence (5'–3') | Annealing Tmp (ºC) | References |
|---|---|---|---|
| Total bacteria | TGGCTCAGGACGAACGCTGGCGGC<br>CCTACTGCTGCCTCCCGTAGGAGT | 61 | 16 |
| *Bacteroides fragilis* group | ATA GCC TTT CGA AAG RAA GAT<br>CCA GTA TCA ACT GCA ATT TTA | 50 | 16, 17 |
| *Clostridium coccoides* group | AAA TGA CGG TAC CTG ACT AA<br>CTT TGA GTT TCA TTC TTG CGA A | 50 | 16, 17 |
| *Clostridium leptum* group | GCA CAA GCA GTG GAG T<br>CTT CCT CCG TTT TGT CAA | 50 | 16, 17 |
| *E. coli* | GTTAATACCTTTGCTCATTGA<br>ACCAGGGTATCTAATCCTGTT | 62 | 18 |
| *Lactobacillus* group | GGAAACAG(A/G)TGCTAATACCG<br>CACCGCTACACATGGAG | 61 | 19, 20 |
| *Bifidobacterium* | CTCCTGGAAACGGGTGG<br>GGTGTTCTTCCCGATATCTACA | 55 | 16, 17 |
| *B. longum* | TTCCAGTTGATCGCATGGTC<br>TCSCGCTTGCTCCCCCGAT | 55 | 16, 17 |
| *B. bifidum* | CCACATGATCGCATGTGATTG<br>CCGAAGGCTTGCTCCCAAA | 55 | 16, 17 |
| *B. breve* | CCGGATGCTCCATCACAC<br>ACAAAGTGCCTTGCTCCCT | 55 | 16, 17 |
| *B. adolescentis* | CTCCAGTTGGATGCATGTC<br>TCCAGTTGACCGCATGGT | 55 | 16, 17 |
| *B. catenulatum* group | CGGATGCTCCGACTCCT<br>CGAAGGCTTGCTCCCGAT | 55 | 16, 17 |

**Table 2. Clinical characteristics of the studied subjects**

| **Total subjects** | **N=36** |
|---|---|
| Age (years) | 14.5 (13.0-15.0) |
| Body mass index (BMI) | |
| before intervention | 32.8 (29.4-35.2) |
| after intervention | 30.6 (27.5-33.3) |
| Weight (kg) | |
| before intervention | 90.5 (81.8-102.2) |
| after intervention | 84.4 (75.3-97.1) |
| Weight loss (kg) | 4.7 (1.7-7.2) |
| Weight loss (%) | 5.8 (2.2-8.6) |
| BMI z-score | |
| before intervention | 3.09 (2.31-4.08) |
| after intervention | 2.71 (1.72-3.49) |

| **Low weight-loss group** | **N=13** |
|---|---|
| Age (years) | 14.5 (13.0-15.0) |
| Body mass index (BMI) | |
| before intervention | 30.7 (26.4-36.3) |
| after intervention | 30.2 (26.2-35.9) |
| Weight (kg) | |
| before intervention | 85.9 (69.4-101.6) |
| after intervention | 84.4 (68.2-100.7) |
| Weight loss (kg) | 1.4 (0.75-1.8) |
| Weight loss (%) | 1.3 (0.85-2.25) |
| BMI z-score | |
| before intervention | 2.95 (1.6 - 4.03) |
| after intervention | 2.74 (1.5-3.93) |

| **High weight-loss group** | **N=23** |
|---|---|
| Age (years) | 14.5 (14.0-15.0) |
| Body mass index (BMI) | |
| before intervention | 33.1 (30.0-35.0)* |
| after intervention | 31.1 (27.5-32.9)* |
| Weight (kg) | |
| before intervention | 92.3 (83.8-102.5) |
| after intervention | 84.7 (77.6-95.4) |
| Weight loss (kg) | 6.9 (4.8-9.3) |
| Weight loss (%) | 7.5 (5.8-9.3) |
| BMI z-score | |
| before intervention | 3.22 (2.57-4.16)* |
| after intervention | 2.67 (1.73-3.30)* |

*Data are shown as medians and interquartile range (IQR). Statistical differences before and after intervention were calculated by using the Mann-Whitney *U*-test at $P < 0.050$

**Table 3.** Daily energy and nutrient intake before (baseline) and after the intervention.

| | Low weight-loss group (> 2.0 kg) | | | | High weight-loss group (> 4.0 kg) | | | |
|---|---|---|---|---|---|---|---|---|
| | Before Intervention (n=13) | | After intervention (n=13) | | Before Intervention (n=23) | | After intervention (n=23) | |
| | Mean | S.D | Mean | S.D | Mean | S.D | Mean | S.D |
| Energy (kcal) [ab] | 2121.67 | 617.00 | 1428.55 | 216.83 | 2377.62 | 617.56 | 1460.62 | 376.52 |
| Water (g) [a] | 1325.40 | 377.84 | 1109.62 | 290.99 | 1912.94 | 657.90 | 1999.0 | 708.51 |
| Protein (g) [ab] | 101.30 | 23.47 | 75.59 | 11.66 | 109.82 | 29.92 | 77.04 | 19.56 |
| Energy from protein (%) [ab] | 18.90 | 3.08 | 22.58 | 2.59 | 18.62 | 3.34 | 21.96 | 2.40 |
| Plant protein (g) [ab] | 29.28 | 8.13 | 20.58 | 4.26 | 26.37 | 7.88 | 22.44 | 6.97 |
| Plant protein (%) [b] | 5.58 | 2.06 | 5.64 | 1.25 | 5.31 | 1.89 | 6.38 | 1.26 |
| Animal protein (g) [b] | 72.32 | 22.55 | 54.10 | 9.97 | 76.56 | 28.67 | 54.67 | 15.37 |
| Animal protein (%) | 12.80 | 3.11 | 16.21 | 2.41 | 13.43 | 6.54 | 15.77 | 2.37 |
| Fat (g) [ab] | 91.66 | 45.82 | 51 | 16.32 | 99.39 | 38.25 | 52.11 | 17.66 |
| Energy from fat (%) [b] | 38.11 | 9.25 | 34.43 | 8.20 | 40.44 | 5.55 | 31.85 | 5.41 |
| Saturated fat (g) [ab] | 26.96 | 18.06 | 17.93 | 4.69 | 36.37 | 15.73 | 15.44 | 6.50 |
| Energy from saturated fat (%)[b] | 11.67 | 3.70 | 10.96 | 2.34 | 13.30 | 3.20 | 9.58 | 2.18 |
| MUFA (g) [ab] | 42.41 | 22.59 | 22.96 | 9.76 | 42.05 | 16.38 | 21.94 | 9.14 |
| Energy from MUFA (%) [b] | 17.65 | 6.27 | 15.52 | 5.72 | 16.62 | 3.35 | 13.45 | 3.68 |
| PUFA (g) [ab] | 13.22 | 6.35 | 7.03 | 3.64 | 16.82 | 8.01 | 7.25 | 2.47 |
| Energy from PUFA (%) [b] | 5.77 | 1.25 | 4.91 | 1.71 | 6.82 | 2.40 | 4.25 | 0.93 |
| Cholesterol (mg) [ab] | 332.01 | 114.01 | 257.97 | 67.94 | 371.80 | 165.12 | 215.16 | 118.79 |
| CH (g) [ab] | 223.03 | 55.40 | 153.71 | 33.32 | 226.29 | 63.37 | 163.63 | 48.75 |
| Energy from CH (%) [b] | 43.30 | 8.36 | 45.03 | 7.38 | 41.89 | 5.62 | 47.28 | 5.07 |
| Simple CH (g) [ab] | 99.85 | 32.80 | 61.99 | 27.63 | 108.36 | 43.83 | 80.91 | 20.37 |
| Energy from simple CH (%) [b] | 16.54 | 6.32 | 21.23 | 7.35 | 18.23 | 5.17 | 22.86 | 6.10 |
| Complex CH (g) [ab] | 114.63 | 38.16 | 74.17 | 21.33 | 114.80 | 32.94 | 81.60 | 36.73 |
| Energy from complex CH (%) | 23.88 | 5.77 | 24.6 | 5.54 | 21.29 | 13.61 | 23.96 | 5.34 |
| Dietary fiber (g) | 18.31 | 7.78 | 17.9 | 4.63 | 17.47 | 7.60 | 21.38 | 7.66 |

Abbreviations: PUFA = Polyunsaturated fatty acids, MUFA = Monounsaturated fatty acids, CH = Carbohydrates

[a] Significant ($p < 0.050$) difference within the low-weight loss group between baseline and after the intervention,

[b]Significant difference within the high-weight loss group (based on age and sex adjusted ANOVA analysis for repeated measurements of log-transformed dietary data).

**Table 4.** Bacterial counts in fecal samples of low weight-loss (<2.0 kg) group of adolescents, before and after intervention

| Bacterial group | Bacterial counts[a] (Log cells/g fecal sample), n=13 | | | | | | | | Mann-Whitney U-test P-Value |
|---|---|---|---|---|---|---|---|---|---|
| | Before intervention | | | | After intervention | | | | |
| | Pr[b] | Mean | Median | IQR | Pr[b] | Mean | Median | IQR | |
| *Total bacteria* | 13 | 13.2 | 12.9 | 12.8-13.9 | 13 | 13.2 | 13.1 | 12.8-13.4 | 0.975 |
| *Bacteroides* | 13 | 6.2 | 6.2 | 5.8-7.0 | 13 | 6.3 | 6.2 | 5.8-6.9 | 0.957 |
| *C.coccoides* | 13 | 10.0 | 10.0 | 9.8-10.2 | 13 | 9.9 | 10.0 | 9.7-10.2 | 0.978 |
| *C.leptum* | 13 | 8.2 | 8.0 | 7.9-8.5 | 13 | 8.4 | 8.3 | 7.9-8.8 | 0.446 |
| *Lactobacillus* | 13 | 7.9 | 7.8 | 7.6-8.1 | 13 | 7.9 | 7.9 | 7.7-8.1 | 0.723 |
| *E.coli* | 13 | 6.7 | 6.5 | 6.0-7.7 | 13 | 6.6 | 6.5 | 6.0-7.1 | 0.624 |
| *Bifidobacterium* | 13 | 9.2 | 9.2 | 8.8-9.5 | 13 | 8.9 | 9.0 | 8.4-9.6 | 0.514 |
| *B.longum* | 13 | 7.1 | 7.0 | 6.8-7.4 | 13 | 7.0 | 6.9 | 6.3-7.7 | 0.644 |
| *B.breve* | 13 | 4.8 | 4.8 | 4.4-5.2 | 13 | 4.5 | 4.5 | 4.3-4.7 | 0.110 |
| *B.bifidum* | 13 | 9.1 | 9.0 | 8.8-9.4 | 13 | 8.9 | 8.9 | 8.3-9.7 | 0.640 |
| *B.adolescentis* | 13 | 8.1 | 8.0 | 7.8-8.4 | 13 | 8.0 | 7.9 | 7.3-8.7 | 0.650 |
| *B.catenolatum* | 13 | 5.8 | 5.8 | 5.5-6.2 | 13 | 5.5 | 5.5 | 5.3-5.7 | 0.103 |

[a]Data are shown as medians and interquartile range (IQR) of cell number per gram of fecal samples

*Statistical differences between bacterial counts before and after intervention were calculated by using the Mann-Whitney *U*-test and established at $P < 0.050$.

[b]Prevalence (Pr) reflects the number of positive amplifications by qPCR from total samples (n=13).

**Table 5.** Bacterial counts in fecal samples of high weight-loss (> 4.0 kg) group of adolescents, before and after intervention.

| Bacterial group | Bacterial counts[a] (Log cells/g fecal sample), (n=23) | | | | | | | | Mann-Whitney *U*-test *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | Before intervention | | | | After intervention | | | | |
| | Pr[b] | Mean | Median | IQR | Pr | Mean | Median | IQR | |
| Total bacteria | 23 | 14.8 | 14.6 | 14.0-15.6 | 23 | 14.5 | 14.8 | 13.1-16.1 | 0.450 |
| *Bacteroides* | 23 | 7.5 | 7.6 | 6.7-8.2 | 23 | 8.6 | 8.6 | 8.1-9.3 | 0.001* |
| *C. coccoides* | 23 | 8.7 | 8.6 | 8.3-9.0 | 23 | 7.9 | 7.7 | 7.4-8.5 | 0.001* |
| *C. leptum* | 23 | 9.5 | 9.6 | 8.7-9.9 | 21 | 9.5 | 9.7 | 9.1-10.0 | 0.666 |
| *Lactobacillus* | 23 | 6.4 | 6.4 | 5.9-6.9 | 23 | 6.9 | 7.0 | 6.3-7.1 | 0.007* |
| *E. coli* | 23 | 6.3 | 6.3 | 5.8-6.8 | 23 | 6.4 | 6.3 | 6.1-7.0 | 0.231 |
| *Bifidobacterium* | 23 | 8.3 | 8.1 | 7.7-8.6 | 23 | 8.2 | 8.2 | 7.4-8.6 | 0.692 |
| *B. longum* | 23 | 7.1 | 7.2 | 6.3-7.9 | 23 | 6.4 | 6.2 | 5.3-7.3 | 0.044* |
| *B. breve* | 15 | 3.5 | 3.3 | 3.0-3.6 | 11 | 3.2 | 3.1 | 3.0-3.5 | 0.237 |
| *B. bifidum* | 19 | 5.9 | 5.6 | 4.5-7.1 | 17 | 5.6 | 5.6 | 4.3-7.1 | 0.490 |
| *B. adolescentis* | 23 | 7.6 | 7.9 | 6.8-8.8 | 23 | 6.9 | 7.0 | 6.0-8.1 | 0.082 |
| *B. catenulatum* | 22 | 7.6 | 7.7 | 6.7-8.5 | 23 | 7.2 | 7.6 | 6.3-8.4 | 0.594 |

[a]Data are shown as medians and interquartile range (IQR) of cell number per gram of fecal samples

*Statistical differences between bacterial counts before and after intervention were calculated by using the Mann-Whitney *U*-test and established at $P < 0.050$.

[b]Prevalence (Pr) reflects the number of positive amplifications by qPCR from total samples (n=23).

**Table 6.** Bacterial counts in fecal samples of low and high weight-loss groups of adolescents, before and after intervention.

| Bacterial group | Bacterial counts[a] before intervention (Log cells/g fecal sample) | | | | | | | | Mann-Whitney *U*-test *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low weight loss group (<2.0 kg) (n=13) | | | | High-weight loss group (>4.0 kg) (n=23) | | | | |
| | Pr[b] | Mean | Median | IQR | Pr | Mean | Median | IQR | |
| Total bacteria | 13 | 13.2 | 12.9 | 12.8-13.9 | 23 | 14.8 | 14.6 | 14.0-15.6 | <0.001* |
| *Bacteroides* | 13 | 6.2 | 6.2 | 5.8-7.0 | 23 | 7.5 | 7.6 | 6.7-8.2 | 0.004* |
| *C. coccoides* | 13 | 10.0 | 10.0 | 9.8-10.2 | 23 | 8.7 | 8.6 | 8.3-9.0 | <0.001* |
| *C. leptum* | 13 | 8.2 | 8.0 | 7.9-8.5 | 23 | 9.5 | 9.6 | 8.7-9.9 | <0.001* |
| *Lactobacillus* | 13 | 7.9 | 7.8 | 7.6-8.1 | 23 | 6.4 | 6.4 | 5.9-6.9 | <0.001* |
| *E. coli* | 13 | 6.7 | 6.5 | 6.0-7.7 | 23 | 6.3 | 6.3 | 5.8-6.8 | 0.123 |
| *Bifidobacterium* | 13 | 9.2 | 9.2 | 8.8-9.5 | 23 | 8.3 | 8.1 | 7.7-8.6 | 0.001* |
| *B. longum* | 13 | 7.1 | 7.0 | 6.8-7.4 | 23 | 7.1 | 7.2 | 6.3-7.9 | 0.845 |
| *B. breve* | 13 | 4.8 | 4.8 | 4.4-5.2 | 15 | 3.5 | 3.3 | 3.0-3.6 | 0.001* |
| *B. bifidum* | 13 | 9.1 | 9.0 | 8.8-9.4 | 19 | 5.9 | 5.6 | 4.5-7.1 | <0.001* |
| *B. adolescentis* | 13 | 8.1 | 8.0 | 7.8-8.4 | 23 | 7.6 | 7.9 | 6.8-8.8 | 0.468 |
| *B. catenulatum* | 13 | 5.8 | 5.8 | 5.5-6.2 | 22 | 7.6 | 7.7 | 6.7-8.5 | 0.030* |

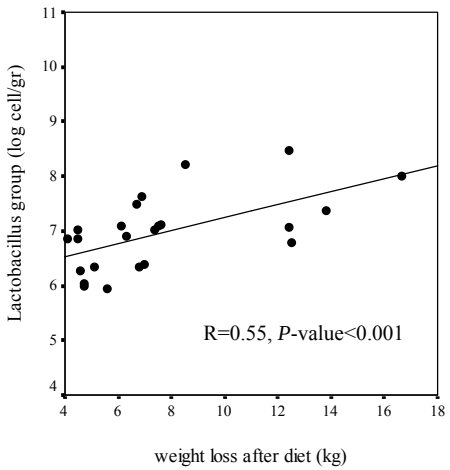
| Bacterial group | Bacterial counts[a] after intervention (Log cells/g fecal sample) | | | | | | | | Mann-Whitney *U*-test *P*-value |
|---|---|---|---|---|---|---|---|---|---|
| | Low weight-loss group (<2.0 kg) (n=13) | | | | High weight-loss group (> 4.0 kg) (n=23) | | | | |
| | Pr[b] | Mean | Median | IQR | Pr | Mean | Median | IQR | |
| Total bacteria | 13 | 13.2 | 13.1 | 12.8-13.4 | 23 | 14.5 | 14.8 | 13.1-16.1 | 0.015* |
| *Bacteroides* | 13 | 6.3 | 6.2 | 5.8-6.9 | 23 | 8.6* | 8.6 | 8.1-9.3 | 0.001* |
| *C. coccoides* | 13 | 9.9 | 10.0 | 9.7-10.2 | 23 | 7.9* | 7.7 | 7.4-8.5 | <0.001* |
| *C. leptum* | 13 | 8.4 | 8.3 | 7.9-8.8 | 21 | 9.5 | 9.7 | 9.1-10.0 | <0.001* |
| *Lactobacillus* | 13 | 7.9 | 7.9 | 7.7-8.1 | 23 | 6.9* | 7.0 | 6.3-7.1 | <0.001* |
| *E. coli* | 13 | 6.6 | 6.5 | 6.0-7.1 | 23 | 6.4 | 6.3 | 6.1-7.0 | 0.972 |
| *Bifidobacterium* | 13 | 8.9 | 9.0 | 8.4-9.6 | 23 | 8.2 | 8.2 | 7.4-8.6 | 0.008* |
| *B. longum* | 13 | 7.0 | 6.9 | 6.3-7.7 | 23 | 6.4* | 6.2 | 5.3-7.3 | 0.062 |
| *B. breve* | 13 | 4.5 | 4.5 | 4.3-4.7 | 11 | 3.2 | 3.1 | 3.0-3.5 | <0.001* |
| *B. bifidum* | 13 | 8.9 | 8.9 | 8.3-9.7 | 17 | 5.6 | 5.6 | 4.3-7.1 | <0.001* |
| *B. adolescentis* | 13 | 8.0 | 7.9 | 7.3-8.7 | 23 | 6.9 | 7.0 | 6.0-8.1 | 0.063 |
| *B. catenulatum* | 13 | 5.5 | 5.5 | 5.3-5.7 | 23 | 7.2 | 7.6 | 6.3-8.4 | 0.036* |

[a]Data are shown as medians and interquartile range (IQR) of cell number per gram of fecal samples

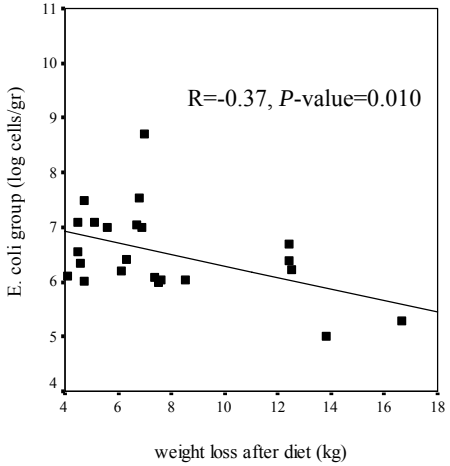
*Statistical differences between bacterial counts for each group (high-weight and low-weight adolescent groups) before and after intervention were calculated by using the Mann-Whitney *U*-test and established at $P < 0.050$.

[b]Prevalence (Pr) reflects the number of positive amplifications by qPCR from total samples (n=13 or 23).

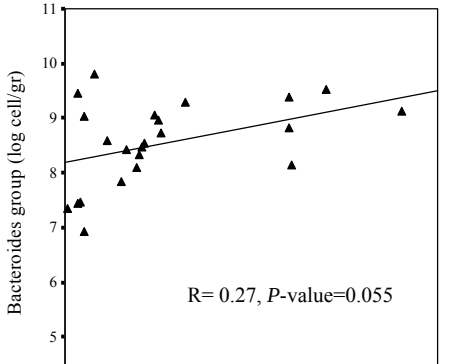
A)


Lactobacillus group (log cell/gr)
R=0.55, P-value<0.001
weight loss after diet (kg)

B)


E. coli group (log cells/gr)
R=-0.37, P-value=0.010
weight loss after diet (kg)

C)


Bacteroides group (log cell/gr)
R= 0.27, P-value=0.055