Iran Red Crescent Med J. 2018 April; 20(4):e58049. doi: 10.5812/ircmj.58049.

Published online 2018 July 31. **Research Article**

Correlation of Gut Microbiota Profile with Body Mass Index Among School Age Children

Seyed Haydeh Mousavi[1], Seyfeddin Mehrara[1], Abolfazl Barzegari[1] and Alireza Ostadrahimi[1,*]

[1]Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran

[*]*Corresponding author*: Nutrition Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. Email: ostadrahimi@tbzmed.ac.ir

Received 2017 September 14; **Revised** 2017 December 22; **Accepted** 2018 January 23.

Abstract

Background: The correlation between gut microbiota with body mass index is controversial. This study aimed to explore the correlation between gut microbiota profiles, Bacteroidetes and Firmicutes, with a body mass index in 7 - 12- year- old school aged children, Iran.

Methods: This cross-sectional study was carried out on school-age children. A total of 188 elementary school children were selected through cluster sampling frame. Data collection tool was the international physical activity questionnaire (IPAQ), therefore, we checked the anthropometric characteristics. Fecal sampling was obtained from all study samples, Langround, Iran. Obese (BMI = 25.8 $\pm$ 3.40 kg/m^2), normal-weight (BMI = 15.54 $\pm$ 1.19 kg/m^2), and lean (BMI = 12.79 $\pm$ 1.8 kg/m^2) among Langroud children aged 7 - 12 years. The total stool bacterial genomic DNA was extracted by quantitative real-time PCR (Q-PCR) to determine the colony forming units (CFU) of Bacteroidetes and Firmicutes. Q_PCR data were analyzed by using SPSS version 19.0, and analyzed interpreted statistical exams such as Spearman's rank correlation coefficient and Kruskal Wallis test. Since data was not normal, $P < 0.05$ was set as a significant level.

Results: Gut microbiota, Firmicutes and Bacteroidetes CFU, and so bact/firm ratio were significantly different among the three group fecal samples ($P < 0.0001$, $P = 0.025$, $P = 0.004$). Bacteroidetes and bact/firm ratio had a significant difference among girls ($P = 0.037$, $P = 0\ 0013$); however, there is no significant difference among boys. The results indicate that there is a significant negative correlation between bact/firm ratio with BMI and waist circumference ($r = -176$, $P = 0.016$, $r = -151$, $P = -0.3$).

Conclusions: The amount of Bacteroidetes and so bact/firm ratio were decreased among obese children; however; Firmicutes increased. It was suggested that obesity in children might be associated with the imbalance of gut microbes.

Keywords: Bacteroidetes, Firmicutes, Gastrointestinal, Gut, Microbiota, Microbiome, Obesity

1. Background

Obesity is defined physiologically as an increase in body fat due to the positive energy balance in the long-term (1). Having an overweight childhood has an epidemic proportion in most developed and developingcountries (2, 3). According to the WHO report (4), more than 155 million children are overweight and obese, of which 42 million are children under the age of five (5).

Obesity is one of the most critical nutritional disorders among Iranian children and adolescents (6). The WHO reported that Iran is one of the seven countries with the highest prevalence of childhood obesity (7).

The ability for obesity depends on the interplay between genetic and environmental factors (8-11).

Therefore, determining and identifying the variables that can be used to reduce obesity is very important (12). Studies have shown that intestinal microbiota, as a link between the gene and environment play an essential role in metabolic regulation, digestion, and food intake (13-15).

The composition of the gut microbiota depends on the age, gender, geographical environment, race, family, and dietand can be changed with probiotics and antibiotics intake (14, 16, 17).

The most abundant bacterial strains in humans and mice are Firmicutes (60% - 80%) and Bacteroidetes (20% - 40%), which are anaerobic chains (14, 18).

Animal studies indicate an increase in the number of Firmicutes and a reduction in Bacteroidetes in obese samples compared to lean and normal mice (14, 19-21).

A recent study suggests that despite the fact there is the link between the intestinal microbiota and obesity, in many animal and laboratory models, human studies have shown controversial results; thus, some of the findings supported a higher proportion of firm/bact in obese subjects (22-24), and many studies did not find a meaningful relationship between firm/bact with BMI (25, 26).

2. Objectives

In order to provide an appropriate way to reduce the prevalence of obesity in children, further studies are needed in more limited populations of age, sex, race, dietary habits, and lifestyle. Guilan is located in the north of Iran and is the second provenance with the highest prevalence of childhood obesity; this is the first study aimed to determine the relationship between gut microbiota with BMI among school-age children in Langroud city, Iran.

3. Methods

3.1. The Participants and the Study Design

This cross-sectional study was carried out in Langroud city, Gilan, Iran. From December 2015 to February 2015.

A total of 188 school age children (ages 7 - 12 years) were selected through cluster framework sampling method.

The inclusion criteria were students with age between 7 to 12 years, and willing to participate in the study. The exclusion criteria included individuals who received antibiotics at least two weeks before fecal collection, severe stresses tolerance that could affect the microbiota, trauma, and severe infection, as well as gastrointestinal problems such as abdominal pain, diarrhea, and constipation.

6360 students that were distributed among 570 classes, were included. Through the cluster sampling method 17 classes were chosen randomly, and 188 fecal samples were analyzed.

Informed consent was obtained from the parents, this study was approved by the ethics committee of Tabriz University of Medical Sciences (TBZMED.REC.1394.1095).

3.2. Measurements and Sample Collection

Data collection tools were two researchers made questionnaires regarding demographic variables and the short form of the international physical activity questionnaire (IPAQ). In the demographic questionnaire, all variables such as age, sex, weight (kg), height (m), BMI (kg/m^2) and waist circumference (cm) were asked. The students' body mass index (BMI) were calculated. The principles to be obese, normal, and lean were WHO (2007) classification growth reference /BMI for ages 5 - 19 (27), which is exact for age and gender.

IPAQ short form is the international physical activity questionnaire with seven questions to determine the measurement properties of these questionnaires. A reliability and validity study was carried out in 14 centers in 12 countries during 2000 (28). The short form of the international physical activity questionnaire (IPAQ) assesses physical activity in the student. The questionnaire encompasses three specific types of activity (moderate-intensity, walking, and vigorous-intensity activity). Data obtained from the first self-administered and scored IPAQ were applied for generating a metabolic equivalent total score (METs), considered by adding MET-min/week for all activities completed. This questionnaire has been used in many studies (29, 30). All data collection was done by one researcher.

Stool samples were collected in sterile boxes, which were delivered to each deliberate students' parents; all fecal samples were received less than two hours after defecation in the early morning and immediately stored at -70°C for further preparation.

3.3. Real-Time PCR

Whole DNA was extracted from the isolated bacteria of gut fecal samples using the QIAamp DNA Stool Mini Kit (Senso Quest Lab Cycler, Germany) and then stowed at -20°C until subsequent use in Q-PCR. Genomic DNA was extracted from *E. coli* and *L. plantarum*. The next serial dilutions of the primitive solution were utilized to make the standard curve: 50 ng, 5 ng, 0.5 ng, 0.05 ng, and 0.005ng. The standard curves were obtained using the IQ-5 Q-PCR noticing system (Bio-rad, USA).

To control the absolute amount of Firmicutes and Bacteroidetes in the gut flora, textbooks (Bioneer) for the traditional order of the 16S r RNA genes of both groups of bacteria were intended. For Firmicutes, using a primer was with the flowing sequence: rFerF: 5'-TCCTACGGGAGGCAGCAGTAG-3'and rFerR: 5'-TACGTATTACCGCGGCTGCTG-3' and for Bacteroidetes prime (was: Bac296F: 5'-GAGAGGAAGGTCCCCCAC-3' All-Bac412R: 5'-CTACTTGGCTGGTTCAG-3'. Q-PCR reactions were denatured at 95°C for 10 minutes and followed by 45 cycles of 95°C for 15 seconds and 62°C for Firmicutes and 60°C Bacteroidetes for 40 seconds, and 72°C for 25 seconds. (ABI biosystem, England; kbp, Ferments, France; Bio-rad, USA). Specificity of the PCR reaction was confirmed by the melting curve, and the copy number of the bacterial gene was calculated using Ct value and the standard curve. Data were presented as the logarithm of copy number (xlgx $\pm$ slgx), and the ratio of Bacteroidetes to Firmicutes (bact /firm) was calculated. All PCR tests were performed in one laboratory by one person (Table 1).

3.4. Statistical Analysis of the Studies

After the Kolmogorov-Smirnov test, all normal distributive variables were examined by t-test. For comparison of means, the one-way ANOVA test, and for non-normal distributive variables some exams such as Kruskal Wallis test, the Mann-Whitney U test, and Chi-square test were used for qualitative variables.

Table 1. Sequences of Primers

Primer	Sequence	Size of Products (bp)	Annealing Tm (°C)	Ref
Firmicutes	rFerF:5'-TCCTACGGGAGGCAGCAGTAG-3'	200	62	This research
	rFerR: 5'-TACGTATTACCGCGGCTGCTG-3'			
Bacteroides	AllBac296F:5'-GAGAGGAAGGTCCCCCAC-3'	106	60	Layton 2006
	AllBac412R: 5'-GCTACTTGGCTGGTTCAG-3'			

Furthermore, Spearman's rank correlation coefficient was used to identify the correlation of Bacteroidetes to Firmicutes ratio with BMI, MET (min/w), and WC (wrist circumference). P-values < 0.05 were considered as significant level. Statistical analyses were done using SPSS 19.0 statistical software (SPSS Inc., Chicago, IL, USA).

4. Results

A total of 188 subjects (90 boys and 98 girls) with a mean age of 9.58 ± 1.43 were registered for evaluation. Subjects were divided into the three groups; obese, normal weight, and lean based upon their BMI. Demographic data (age, sex, BMI, WC, MET) were shown in Table 2.

Among the groups, there were significant differences in BMI, WC, and Age (P < 0.05).

4.1. The Q-PCR and Analysis of Molecular Data

Significant difference in Firmicutes CFU, Bacteroidetes, and bact/firm ratio (P < 0.0001, P = .025, and P = .004) among the collections were recognized respectively (Table 3). Gender differences were experiential in Bacteroidetes CFU, Firmicutes CFU, and bact/firm in groups (lean, normal weight, and obese). We showed that there was a significant difference in Bacteroidetes CFU, Firmicutes CFU, and bact/firm ratio in the girls of all groups (P = 0.002, P = 0.037, and P = 0.013). There was also a significant difference in Firmicutes CFU (P = 0.0002) in the boys of all groups, however, for Bacteroidetes and bact/firm ratio, there was no significant difference. We reported that Firmicutes CFU in both lean and obese boys (P = 0.0001, P = 0.011) and girls (P = 0.005, P = 0.002) was higher than that in normal cases. Results showed that bact/firm ratio in normal girls (P = 0.004) was significantly higher than that of obese girls, however, this difference was not significant in boys. Additionally, the Bacteroidetes CFU (P = 0.005) in lean girls was significantly higher than that of obese girls, however, it was not significant in other groups (Table 3).

Results indicate that there is a significant negative correlation between bact/firm ratio with BMI and waist circumference (r = -176, P = 0.016, r = -151, P = 0.03). However, the results for other indicators such as age, sex, and physical activity (31) were not significant (Table 4).

5. Discussion

This study aimed to assess the likely difference of the gut microbiota; Bacteroidetes and Firmicutes, in obese, normal weight, and lean among students in Langroud elementary schools age children. Results showed that the number of Bacteroidetes was apparently lower in obese children than those in lean one. Bact/firm ratio was notably lower in obese children than that in normal weight children, which is in concordance with those reported by Xu et al. (11), Turnbaugh et al. (13), Ley et al. (17), Li et al. (32), Borgo et al. (33), Kasai et al. (34) and Riva et al. (35). However, many studies have shown contradictory results, for example, Duncan et al. (24), showed that the CFU of Bacteroidetes in obese kid subjects were higher compared with those in normal weight, and the quantity of Bacteroidetes continued to remain unchanged in the next control diet in fat contributors (24). Also Ochoa-Acosta et al. (36), showed no significant differences in the structure and status of microbiota among lean and obese Mexican women. In addition, the results showed that there was a negative correlation between bact/firm ratios with BMI. This finding was also indicated by Ley et al. (17), Xu et al. (11), and Ignacio et al. (37). Jumpertz et al. (38), showed that the reduced Bacteroidetes and increased Firmicutes were correlated with obesity.

Langroud people are relatively an isolated minority in Iran and have similarities in the living environment and diet, which would probably minimalize the change in gut microbiota among students.

The current study also showed that the differences in the number of Bacteroidetes were experiential between obese and lean girls; however, not among boys. This is dissimilar to what has been stated by Mueller et al. (39), who reported a higher Bacteroidetes population in male than in the female, a result that may be clarified by the age and geographical area differences between these two studies.

We reported that differences in Firmicutes CFU in both obese boys and girlswere greater than that in normal cases, however, it was not significant in lean and obese boys and girls. This finding is different from that reported by Xu et al. (11), who reported that there was no significant difference between the number of Firmicutes in obese, normal

Table 2. Demographic Information, Characteristics, and Microbiota Data of the Subjects Separated by Group[a,b,c]

Variation	Lean (1)	Normal (2)	Obese (3)	Total	P (1,2,3)	P (1, 2)	P (1, 3)	P (2, 3)
Age (y)	9.31 ± 1.46	9.26 ± 1.29	10.14 ± 1.39	9.58 ± 1.43	> 0.0001[d]	0.952	0.002[d]	0.0001[d]
Boy	31.0 (51.7)	29.0 (46.0)	30.0 (46.2)	90.0 (47.9)	0.775	-	-	-
Girl	29.0 (48.3)	34.0 (54.0)	35.0 (53.8)	98.0 (52.1)				
WC (cm)	54.9 ± 3.0	59.5 ± 4.6	78.1 ± 9.3	64.5 ± 11.9	> 0.0001	0.0001[d]	0.0001[d]	0.0001[d]
BMI (kg/m²)	12.79 ± .81	15.54 ± 1.19	25.80 ± 3.40	18.21 ± 6.04	> 0.0001[d]	0.0001[d]	0.0001[d]	0.0001[d]

Abbreviations: BMI, body mass index; WC, wrist circumference.
[a] (N = 188) data were presented as mean ± SD for normal data and No. (%) for categories.
[b] Differences among two groups were compared using t-test for normal data and Chi-square for qualitative variables.
[c] Differences among three groups were compared using ANOVA because the data was not normal.
[d] $P < 0.05$, indicates significant differences among groups.

Table 3. Univariate Analysis of the Differences Between Bacteroidetes and Firmicutes with BMI Levels by Gender[a]

	Lean (1) (n = 60)	Normal (2) (n = 63)	Obese (3) (n = 65)	Total (n = 188)	P (1, 2, 3)	P (1, 2)	P (1, 3)	P (2, 3)
Boy								
Firmicutes (mean CT)	18.46 (17.23 - 23.43)	15.54 (14.54 - 18.54)	18.84 (15.61-24.39)	17.54 (15.48 - 22.21)	0.002[b]	0.0001	0.708	0.011
Bacteroidetes (mean CT)	23.02 (17.74 - 29.19)	19.22 (16.75 - 24.42)	18.90 (15.43 - 26.16)	20.02 (16.55 - 26.61)	0.260	0.149	0.168	0.844
Bact/firm	1.08 (0.83 - 1.56)	1.21 (1.01 - 1.58)	0.97 (0.83 - 1.36)	1.09 (0.87 - 1.54)	0.248	0.387	0.471	0.087
Girl								
Firmicutes (mean CT)	19.54 (18.33 - 27.43)	16.88 (15.22 - 20.53)	21.51 (17.42 - 27.34)	19.10 (16.14 - 24.32)	0.002[b]	0.005	0.925	0.002
Bacteroidetes (mean CT)	25.04 (19.66 - 28.13)	20.31 (17.01 - 27.92)	20.19 (17.17 - 22.41)	20.80 (17.18 - 27.03)	0.037[b]	0.341	0.005[b]	0.240
Bact/firm	1.18 (0.83 - 1.57)	1.20 (0.89 - 1.65)	0.90 (0.73 - 1.14)	1.04 (0.83 - 1.51)	0.013[b]	0.356	0.074	0.004
Total								
Firmicutes (mean CT)	19.09 (17.34 - 25.93)	16.11 (14.65 - 19.32)	19.53 (17.24 - 25.43)	18.50 (15.58 - 23.29)	< 0.0001[b]	0.0001[b]	0.974	0.0001
Bacteroidetes (mean CT)	24.95 (18.10 - 28.18)	19.94 (16.75 - 26.54)	20.15 (16.61 - 24.81)	20.33 (16.85 - 26.82)	0.025[b]	0.92	0.006[b]	0.391
Bact/firm	1.12 (0.83 - 1.56	1.21 (0.93 - 1.60)	0.93 (0.78 - 1.27)	1.07 (0.84 - 1.51)	0.004[b]	0.220	0.058	0.0001

[a] Data were presented as median (percentile 25-percentile 75); Differences among three groups were compared using Kruskal-Wallis test and between two groups were compared using the Mann-Whitney U test because data were not normally distributed.
[b] $P < 0.05$, indicates significant differences among groups.

weight, and lean girls and boys.

More studies with more sample sizes are needed to explain the precise character of gut microbiota in the pathogenesis of fatness as well as the effect of gender on microbiota balances. Though fitness is strongly related to the variations in the balance as well as the function of gut microbiota, the mechanism behind this alteration is left over to be clarified. The effect of human microbiota on energy reaping and metabolism has been stated as pathways to clarify their possible relationship with obesity (14). Otherwise, disrupted gut microbiota may change the contact with obesogenic and diabetogenic environmental chemicals (40).

The current study has some limitations. Considering the potential impact of lifestyle on the gut microbiota, it is necessary to make a separated study of the city and the village in the future. Additionally two main phyla of bacteria, Bacteroidetes and Firmicutes, were measured in the feces of Langroud school- age children; however, definite species were not isolated, and each of these phyla contains hundreds or thousands of species with changed metabolic properties; all these organisms can affect the metabolism of food and host physiology. Therefore, the study of some bacteria, specifically probiotics, Bifidobacteria, Lactobacillus, Clostridium, and so on. is essential for the possible probiotic therapy of children in the risk of fatness. Finally, the

Table 4. Partial Correlation Coefficients (r) for the Association Between Demographic Information, Characteristics, and of the and Features of the Bact/Firm Subjects Separated by Group (N = 188)[a]

	Bact/Firm			
	r	P-Value	95% Confidence Interval	
			Lower	Upper
Age (y)	0.111	0.128	-0.242	0.03
Gender	-0.048	0.511	-0.202	0.101
BMI (kg/m^2)	-0.151[b]	0.039	-0.288	0.000
WC (cm)	-0.176[b]	0.016	-0.303	-0.033
Physical activity (METs)	0.129	0.77	-0.017	0.262

Abbreviations: BMI, body mass index; WC, waist circumference.
[a] Spearman analysis was used to investigate the relationship between the ratio of bact/firm with individual characteristics (gender, age, waist circumference, BMI, and physical activity) because the data were not normal.
[b] Correlation is significant at the 0.05 level (2-tailed).

mechanism of gut microbiota on BMI was not evaluated in this study. It is necessary to be considered in the future studies.

5.1. Conclusions

This survey revealed a significant decline in the number of Bacteroidetes and bact/firm ratio and an increase in the number of Firmicutes in the feces of the obese in comparison with normal weight children. Additionally, the number of Bacteroidetes in obese girls was significantly lower than that in lean girls, however, it was not significant in other groups.

According to the fact that being overweight during childhood is an epidemic problem in most developed and developing countries and the result showed that there is a negative correlation between bact/firm with BMI. Further studies on the optimal composition of intestinal microbiota and its ways of maintaining balance can be an essential factor in maintaining health and preventing obesity.

Acknowledgments

We would like to thank all the students and their parents who completed the questionnaire and participated in this study.

Footnote

Conflict of Interests: The authors revealed that there is no conflict of interests.

References

1. Haslam DW, James WP. Obesity. *Lancet*. 2005;**366**(9492):1197–209. doi: 10.1016/S0140-6736(05)67483-1. [PubMed: 16198769].
2. Dehghan M, Akhtar-Danesh N, Merchant AT. Childhood obesity, prevalence and prevention. *Nutr J*. 2005;**4**:24. doi: 10.1186/1475-2891-4-24. [PubMed: 16138930]. [PubMed Central: PMC1208949].
3. Wang Y, Lobstein T. Worldwide trends in childhood overweight and obesity. *Int J Pediatr Obes*. 2006;**1**(1):11–25. [PubMed: 17902211].
4. World Health Organization. *Obesity and Overweight*. World Health Organization; 2013, [updated 16 February 2018]. Available from: http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.
5. Hossain P, Kawar B, El Nahas M. Obesity and diabetes in the developing world–a growing challenge. *N Engl J Med*. 2007;**356**(3):213–5. doi: 10.1056/NEJMp068177. [PubMed: 17229948].
6. Kelishadi R, Pour MH, Sarraf-Zadegan N, Sadry GH, Ansari R, Alikhassy H, et al. Obesity and associated modifiable environmental factors in Iranian adolescents: Isfahan Healthy Heart Program - Heart Health Promotion from Childhood. *Pediatr Int*. 2003;**45**(4):435–42. [PubMed: 12911481].
7. Silventoinen K, Sans S, Tolonen H, Monterde D, Kuulasmaa K, Kesteloot H, et al. Trends in obesity and energy supply in the WHO MONICA Project. *Int J Obes Relat Metab Disord*. 2004;**28**(5):710–8. doi: 10.1038/sj.ijo.0802614. [PubMed: 15007395].
8. Hill JO. Understanding and addressing the epidemic of obesity: an energy balance perspective. *Endocr Rev*. 2006;**27**(7):750–61. doi: 10.1210/er.2006-0032. [PubMed: 17122359].
9. Ho-Pham LT, Lai TQ, Nguyen ND, Barrett-Connor E, Nguyen TV. Similarity in percent body fat between white and Vietnamese women: implication for a universal definition of obesity. *Obesity (Silver Spring)*. 2010;**18**(6):1242–6. doi: 10.1038/oby.2010.19. [PubMed: 20150903].
10. Saulnier DM, Kolida S, Gibson GR. Microbiology of the human intestinal tract and approaches for its dietary modulation. *Curr Pharm Des*. 2009;**15**(13):1403–14. [PubMed: 19442165].
11. Xu P, Li M, Zhang J, Zhang T. Correlation of intestinal microbiota with overweight and obesity in Kazakh school children. *BMC Microbiol*. 2012;**12**:283. doi: 10.1186/1471-2180-12-283. [PubMed: 23190705]. [PubMed Central: PMC3543275].
12. Freedman DS, Dietz WH, Srinivasan SR, Berenson GS. The relation of overweight to cardiovascular risk factors among children and adolescents: the Bogalusa Heart Study. *Pediatrics*. 1999;**103**(6 Pt 1):1175–82. [PubMed: 10353925].
13. Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, et al. A core gut microbiome in obese and lean twins. *Nature*. 2009;**457**(7228):480–4. doi: 10.1038/nature07540. [PubMed: 19043404]. [PubMed Central: PMC2677729].

14. Backhed F, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, et al. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci U S A*. 2004;**101**(44):15718–23. doi: 10.1073/pnas.0407076101. [PubMed: 15505215]. [PubMed Central: PMC524219].
15. Turnbaugh PJ, Gordon JI. The core gut microbiome, energy balance and obesity. *J Physiol*. 2009;**587**(Pt 17):4153–8. doi: 10.1113/jphysiol.2009.174136. [PubMed: 19491241]. [PubMed Central: PMC2754355].
16. Angelakis E, Armougom F, Million M, Raoult D. The relationship between gut microbiota and weight gain in humans. *Future Microbiol*. 2012;**7**(1):91–109. doi: 10.2217/fmb.11.142. [PubMed: 22191449].
17. Ley RE, Turnbaugh PJ, Klein S, Gordon JI. Microbial ecology: human gut microbes associated with obesity. *Nature*. 2006;**444**(7122):1022–3. doi: 10.1038/4441022a. [PubMed: 17183309].
18. Zoetendal EG, Vaughan EE, de Vos WM. A microbial world within us. *Mol Microbiol*. 2006;**59**(6):1639–50. doi: 10.1111/j.1365-2958.2006.05056.x. [PubMed: 16553872].
19. Turnbaugh PJ, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature*. 2006;**444**(7122):1027–31. doi: 10.1038/nature05414. [PubMed: 17183312].
20. Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci U S A*. 2005;**102**(31):11070–5. doi: 10.1073/pnas.0504978102. [PubMed: 16033867]. [PubMed Central: PMC1176910].
21. Cani PD, Delzenne NM. Gut microflora as a target for energy and metabolic homeostasis. *Curr Opin Clin Nutr Metab Care*. 2007;**10**(6):729–34. doi: 10.1097/MCO.0b013e3282efdebb. [PubMed: 18089955].
22. Santacruz A, Collado MC, Garcia-Valdes L, Segura MT, Martin-Lagos JA, Anjos T, et al. Gut microbiota composition is associated with body weight, weight gain and biochemical parameters in pregnant women. *Br J Nutr*. 2010;**104**(1):83–92. doi: 10.1017/S0007114510000176. [PubMed: 20205964].
23. Million M, Maraninchi M, Henry M, Armougom F, Richet H, Carrieri P, et al. Obesity-associated gut microbiota is enriched in Lactobacillus reuteri and depleted in Bifidobacterium animalis and Methanobrevibacter smithii. *Int J Obes (Lond)*. 2012;**36**(6):817–25. doi: 10.1038/ijo.2011.153. [PubMed: 21829158]. [PubMed Central: PMC3374072].
24. Duncan SH, Lobley GE, Holtrop G, Ince J, Johnstone AM, Louis P, et al. Human colonic microbiota associated with diet, obesity and weight loss. *Int J Obes (Lond)*. 2008;**32**(11):1720–4. doi: 10.1038/ijo.2008.155. [PubMed: 18779823].
25. Zhang H, DiBaise JK, Zuccolo A, Kudrna D, Braidotti M, Yu Y, et al. Human gut microbiota in obesity and after gastric bypass. *Proc Natl Acad Sci U S A*. 2009;**106**(7):2365–70. doi: 10.1073/pnas.0812600106. [PubMed: 19164560]. [PubMed Central: PMC2629490].
26. Payne AN, Chassard C, Zimmermann M, Muller P, Stinca S, Lacroix C. The metabolic activity of gut microbiota in obese children is increased compared with normal-weight children and exhibits more exhaustive substrate utilization. *Nutr Diabetes*. 2011;**1**. e12. doi: 10.1038/nutd.2011.8. [PubMed: 23154580]. [PubMed Central: PMC3302137].
27. Duggan MB. Anthropometry as a tool for measuring malnutrition: impact of the new WHO growth standards and reference. *Ann Trop Paediatr*. 2010;**30**(1):1–17. doi: 10.1179/146532810X12637745451834. [PubMed: 20196929].
28. Craig CL, Marshall AL, Sjostrom M, Bauman AE, Booth ML, Ainsworth BE, et al. International physical activity questionnaire: 12-country reliability and validity. *Med Sci Sports Exerc*. 2003;**35**(8):1381–95. doi: 10.1249/01.MSS.0000078924.61453.FB. [PubMed: 12900694].
29. Bashiri Moosavi F, Farmanbar R, Taghdisi M, Atrkar Roshan Z. [Level of Physical Activity among Girl High School Students In Tarom County and Relevant Factors]. *Iran J Health Educ Health Promot*. 2015;**3**(2):133–40. Persian.
30. Hazavehei SMM, Asadi Z, Hassanzadeh A, Shekarchizadeh P. [Comparing the effect of two methods of presenting physical education Π course on the attitudes and practices of female Students towards regular physical activity in Isfahan University of Medical Sciences]. *Iran J Med Educ*. 2008;**8**(1):121–31. Persian.
31. Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K, et al. Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature*. 2012;**488**(7413):621–6. doi: 10.1038/nature11400. [PubMed: 22914093]. [PubMed Central: PMC3553221].
32. Li M, Wang B, Zhang M, Rantalainen M, Wang S, Zhou H, et al. Symbiotic gut microbes modulate human metabolic phenotypes. *Proc Natl Acad Sci U S A*. 2008;**105**(6):2117–22. doi: 10.1073/pnas.0712038105. [PubMed: 18252821]. [PubMed Central: PMC2538887].
33. Borgo F, Verduci E, Riva A, Lassandro C, Riva E, Morace G, et al. Relative Abundance in Bacterial and Fungal Gut Microbes in Obese Children: A Case Control Study. *Child Obes*. 2017;**13**(1):78–84. doi: 10.1089/chi.2015.0194. [PubMed: 27007700].
34. Kasai C, Sugimoto K, Moritani I, Tanaka J, Oya Y, Inoue H, et al. Comparison of the gut microbiota composition between obese and non-obese individuals in a Japanese population, as analyzed by terminal restriction fragment length polymorphism and next-generation sequencing. *BMC Gastroenterol*. 2015;**15**:100. doi: 10.1186/s12876-015-0330-2. [PubMed: 26261039]. [PubMed Central: PMC4531509].
35. Riva A, Borgo F, Lassandro C, Verduci E, Morace G, Borghi E, et al. Pediatric obesity is associated with an altered gut microbiota and discordant shifts in Firmicutes populations. *Environ Microbiol*. 2017;**19**(1):95–105. doi: 10.1111/1462-2920.13463. [PubMed: 27450202]. [PubMed Central: PMC5516186].
36. Ochoa-Acosta DA, Lopez-Flores ME, Osuna-Ramirez I, Gomez-Gil B, Vergara-Jimenez MJ. Association Between Gut Microbiota Composition, Diet, and Anthropometric risk Factor's for Metabolic Disorders in Mexican Women with Food Insecurity. *FASEB J*. 2017;**31**(1_supplement):965.43.
37. Ignacio A, Fernandes MR, Rodrigues VAA, Groppo FC, Cardoso AL, Avila-Campos MJ, et al. Correlation between body mass index and faecal microbiota from children. *Clin Microbiol Infec*. 2016;**22**(3):258. e1–8.
38. Jumpertz R, Le DS, Turnbaugh PJ, Trinidad C, Bogardus C, Gordon JI, et al. Energy-balance studies reveal associations between gut microbes, caloric load, and nutrient absorption in humans. *Am J Clin Nutr*. 2011;**94**(1):58–65. doi: 10.3945/ajcn.110.010132. [PubMed: 21543530]. [PubMed Central: PMC3127503].
39. Mueller S, Saunier K, Hanisch C, Norin E, Alm L, Midtvedt T, et al. Differences in fecal microbiota in different European study populations in relation to age, gender, and country: a cross-sectional study. *Appl Environ Microbiol*. 2006;**72**(2):1027–33. doi: 10.1128/AEM.72.2.1027-1033.2006. [PubMed: 16461645]. [PubMed Central: PMC1392899].
40. Snedeker SM, Hay AG. Do interactions between gut ecology and environmental chemicals contribute to obesity and diabetes? *Environ Health Perspect*. 2012;**120**(3):332–9. doi: 10.1289/ehp.1104204. [PubMed: 22042266]. [PubMed Central: PMC3295356].