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# Gut Microbiota-Obesity Relationship

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**ABSTRACT**

Recently gut microbiota became implicated in the pathogenesis of obesity. Gut microbiota not only digest food but also regulate our immune system, produce vitamins and aggravate insulin resistance. Firmicutes and Bacteroidetes are the dominant bacteria, accounting for approximately 99% of the whole microbiota. To clarify the relationship between obesity and gut microbiota (bacteriodetes and firmicutes types). This study was done in Mansoura Specialized Hospital, Mansoura University during the period from May 2019 to May 2020. Two hundreds patients were included and divided according to body mass index (BMI) into three groups (ideal body weight group, overweight group, and obesity group). All included subjects will undergo history and clinical examination, abdominal ultrasound for obese group, measure body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR). Blood samples analyzed for complete blood lipogram, liver functions, and fasting blood glucose level, CRP. Moreover, stool sample for microbiota study (Bacteroidetes and Firmicutes) by polymerase chain reaction (PCR). There was higher microbiota in obese group than ideal and overweight. Firmicutes was associated with NAFLD, higher CRP, ALT and bilirubin. Higher BMI and lower TC were associated with increased likelihood of exhibiting microbiota with sensitivity 87.9%, specificity 45.5%, PPV 72.2%, and NPV 70%. Microbiota mostly play an important role in pathogenesis of obesity and its complications including metabolic syndrome and fatty liver especially Firmicutes type. This can be possible strategies for treatment of obesity.



## 1. INTRODUCTION

Obesity and its associated disorders have reached an alarming stage worldwide. The last decades have experienced an exponential increase in the number of people suffering from obesity and its associated disorders such as type 2 diabetes (T2D) [1]. Sedentary lifestyle and increased food consumption has been considered the main underlying causes for this obesity epidemic [2]. Environmental and genetic factors have also been implicated including changes in the gut microbiota to play a role in the development of metabolic disorders [3]. Gut microbiota describes all organisms living in the gastrointestinal (GI) tract. The majority of these organisms reside in the large intestine. These bacteria play important physiological role in vital processes such as digestion, vitamin synthesis and metabolism amongst others. Even though the exact mechanism linking gut microbiota to obesity is far from being very well understood, it's well established that

gut microbiota can increase energy production from diet, contribute to low-grade inflammation and regulate fatty acid tissue composition [4]. These processes as well as others have been proposed as the link between obesity and gut microbiota. However, the exact contribution of gut microbiota to the development of obesity and diabetes is not very clear due to many reasons including the complexity and diversity of gut microbes, ethnic variation in studied populations and large variations between individuals studied [5].

Non etheless, modulation of gut microbiota holds a tremendous therapeutic potential to treat the growing obesity epidemic especially when combined with diet and exercise [6].

### *1.1 Aim of the study*

The aim in the present study was to clarify the relationship between obesity and gut microbiota (bacteriodetes and firmicutes types).

## 2. Patients and methods

The study is a case –control study. It was done in Mansoura Specialized Hospital, Mansoura University during the period from May 2019 to May 2020. Two hundreds patients were included and divided according to body mass index (BMI) into three groups (ideal body weight group, overweight group, and obesity group).

### *2.1 Patient collected*

The included subjects were above 18 years old. The exclusion criteria were previous antibiotics use within last two weeks, presence of autoimmune diseases, hepatic or renal diseases and presence of hepatitis C immunoglobulin G or hepatitis B surface antigen.

The study will be approved by Mansoura Faculty of Medicine Ethical Committee and approval will be obtained from each subject.

All included subjects were subjected to complete history taking, clinical examination and abdominal ultrasound for obese group.

For each subject there were measure for weight, height, body mass index (BMI), waist circumference (WC), and waist-to-hip ratio (WHR).

Blood samples were withdrawn from each participating subjects and were analyzed for complete blood lipogram including HDL, total cholesterol, triglycerides and LDL, complete liver functions including alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, and albumin and fasting blood glucose level. By auto analyzer (Dialab - Austria). Determination of high sensitive CRP concentrations will be performed by enzyme linked immunosorbant assay (ELISA) (Quantikine-kit) with sensitivity to 0.022 ng/mL; /l. Moreover, stool sample will be provided by each subject in clean container for microbiota study by polymerase chain reaction (PCR).

Microbiota Study for Bacteroidetes and Firmicutes by PCR

### *2.2 DNA extraction*

Stool sample were emulsified by phosphate buffer and part of emulsification were used for DNA extraction by commercial extraction DNA kit supplied from Promega according to the manufacturer’s instructions. Extracted DNA were kept at –20°C for detection of Bacteroidetes and Firmicutes by PCR.

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### *2.3 Polymerase chain reaction*

The PCR method were performed according to methods published previously. The primers sequences and the amplifications base pairs sizes are summarized in table 1. [7- 9].

Polymerase chain reaction amplification were carried out in 50 microns (µl) reaction mixture containing10 µl of DNA, 5 µl of 10 × PCR buffer, 4 µl of MgCl2 (25 mM), 0.5 µl (5 U/µl) of amplification mixture supplied from Promega 1 µl of each primer with concentration 50 pmol/µl and 4 µl of dNTPs with concentration 10 mM and 24.5 µl of sterile distilled water. The PCR amplification profile include an initial denaturation step of 5 min at 95°C followed by 40cycles of denaturation at 94°C for 45 s, annealingat 62°C for 45 second for Bacteroidetes amplification. The PCR for Firmicutes were an initial denaturation step for 5 minutes at 95C and the annealing were at 58°C at 45 seconds and extension at 72°C for 1.5 min, with a final extension step of 10 min at 72°C. The PCR thermal cycling were done by the use of Biosystem. Gel electrophoresis will be performed by the use of - 1.5% agarose gel in Tris acetate/EDTA (TAE) buffer by using 100 V for 1 hour, and the PCR Bands were visualized by staining with0.01% ethidium bromide (0.5 µg/ml).

**Table (1):** Microbiota species, sequences of the primers and products bp.

| Bacterial Species | Sequences of the Primers | Bp |
|---|---|---|
| Bacteroidetes | AACGCTAGCTACAGGCTTAACA<br>ACGCTACTTGGCTGGTTCA ( | bp396 |
| Firmicutes | GCGTGAGTGAAGAAGT<br>CTACGCTCCCTTTACAC | 161 |

### *2.4 Statistical analysis*

Data were entered and analyzed using IBM-SPSS software (Version 25.0). Qualitative data were expressed as absolute frequency (N) and percentage (%). Quantitative data were initially tested for normality using Shapiro-Wilk's test with data being normally distributed if p>0.050. Presence of significant outliers (extreme values) was tested for by inspecting boxplots. Quantitative data were expressed as Mean ± standard deviation if normally distributed or Median ($25^{th}$ – $75^{th}$ percentiles) if not normally distributed. Chi-Square or Fisher's exact test was used to compare categorical data. Quantitative data between two groups were compared by Independent-Samples t-Test if normally distributed or Mann-Whitney U-test if not. Quantitative data between more than two groups were compared by One-Way ANOVA-Test if normally distributed or Kruskal Wallis H-test if not. Spearman's correlation was used to assess the association between quantitative data while Point biserial Correlation was used to assess the association between dichotomous and quantitative data. For any of the used tests, results were considered as statistically significant if p value ≤ 0.050. Appropriate charts were used to graphically present the results whenever needed.

## 3. Results

**Table (1):** Microbiota incidence in groups of different BMI:

| **Microbiota** | **BMI Group** | | | **$\chi^2$** | **P value** |
|---|---|---|---|---|---|
| | **Ideal weight (n=32)** | **Overweight (n=58)** | **Obese (n=111)** | | |
| Not detected | 18 (56.25%) a | 33 (56.9%) a | 26 (23.4%) b | 23.245 | **<0.0005** |
| Detected | 14 (43.75%) a | 25 (43.1%) a | 85 (76.6%) b | | |
| | **BMI vs. type of microbiota** | | | | |

| **Number of patients** | **14** With microbiota | **25** With microbiota | **85** With microbiota | | |
|---|---|---|---|---|---|
| Bacteroidetes | 6 (42.9%%) a | 18 (72%) a | 42 (49.4%) a | 4.641 | 0.098 |
| Firmicutes | 8 (57.1%) a | 7 (28%) a | 43 (50.5%) a | | |

Data expression: Frequency (%). P: Chi-Square Test. Comparisons of column proportions: Shown as letters (if similar = Insignificant difference, if different = significant difference).

This table shows a statistically significant higher proportion of microbiota (Bacteroidetes / Firmicutes) in obese patients as compared to individuals with ideal weight and those who are overweight.

It shows also no statistically significant difference in the proportions of the two detected microbiota types separately either Bacteroidetes or Firmicutes between the three study BMI groups.

**Table (2):** Comparisons between those with and without detected microbiota:

| Parameter | Group | | Test of significance | |
|---|---|---|---|---|
| | Microbiota detected (n=124) | Microbiota not detected (n=77) | $\chi^2$/t/ Z | P value |
| Sex: Male / Female | 94 (75.8%)/30 (24.2%) | 60 (77.9%)/17 (22.1%) | $\chi^2$ = 0.119 | *0.730* |
| NAFLD by US | 14 (11.3%) | 5 (6.5%) | $\chi^2$ = 1.277 | *0.258* |
| Metabolic syndrome | 34 (27.4%) | 10 (13%) | $\chi^2$ = 5.787 | ***0.016*** |
| Age | 54.39 ± 9.65 | 54.36 ± 9.56 | t= -0.017 | $0.987** |
| Weight kg | 85 (76-94) | 78 (70-88) | Z = -3.290 | **0.001** |
| Height cm | 166 (160-172) | 167 (163-170) | Z = -1.171 | 0.242 |
| BMI | 31= -1.416.4 (27-32.8) | 27 (25.1-30.5) | Z = -4.112 | **<0.0005** |
| WC cm | 86 (81-91) | 82 (77-87) | Z = -3.420 | **0.001** |
| HC cm | 100 (90-101.5) | 99 (88-100) | Z = -3.132 | **0.002** |
| WHR | 0.89 (0.82-0.93) | 0.88 (0.85-0.91) | Z = -0.272 | 0.786 |
| TC mg/dL | 201 (168-234) | 202 (181-268) | Z = -1.629 | 0.103 |
| LDL mg/dL | 127 (116-147.5) | 127 (120-140) | Z = -0.495 | 0.620 |
| HDL mg/dL | 40 (33.5-48) | 39 (35-50) | Z = -0.127 | 0.899 |
| TG mg/dL | 118 (82-172) | 124 (93-157) | Z = -0.279 | 0.780 |
| RBG mg/dL | 87.43 ± 22.55 | 85.36 ± 22.35 | t= -0.633 | $0.528** |
| CRP mg/L | 159 (147-177) | 150 (150-150) | Z = -2.959 | **0.003** |
| AST IU/L | 30 (27-39) | 29 (22.7-35) | Z = -2.131 | **0.033** |
| ALT IU/L | 30 (28-39) | 29 (23-34) | Z | 0.157 |
| AST/ALT ratio | 1 (0.881-1.182) | 1 (0.880-1.179) | Z = -0.007 | 0.994 |
| Bilirubin mg/dL | 0.8 (0.7-0.9) | 0.8 (0.7-0.9) | Z = -0.943 | 0.346 |
| Albumin g/dL | 4 (4-4.2) | 4 (4-4.2) | Z = 1.109 | 0.267 |

P value: Mann-Whitney U test, *Chi-Square test, and **Independent-Samples t-test.

(NAFLD: non-alcoholic fatty liver disease, US: ultrasound, BMI: body mass index, WC: waist circumference, HC: hip circumference, WHR: waist-hip circumference, TC: total cholesterol, LDL: low-density lipoprotein, HDL: high-density lipoprotein, TG: triglyceride, RBG: random blood glucose, CRP: C - reactive protein, AST: Aspartate aminotransferase, ALT: alanine aminotransferase)

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This table shows a statistically significant higher proportion of metabolic syndrome and statistically significantly higher levels of weight, BMI, WC, HC, CRP, and AST in those with detected microbiota as compared to those with no detected microbiota.

**Table (3):** Comparisons between those with Bacteroidetes and Firmicutes microbiota:

| Parameter | Group | | Test of significance | |
|---|---|---|---|---|
| | Bacteroidetes (n=66) | Firmicutes (n=58) | $\chi^2$/t/ Z | P value |
| Sex: Male / Female | 48 (72.7%)/18 (27.3%) | 46 (79.3%)/12 (20.7%) | $\chi^2$ = 0.729 | 0.393 |
| NAFLD by US | 1 (1.5%) | 13 (22.4%) | $\chi^2$ = 13.462 | **<0.0005** |
| Metabolic syndrome | 19 (28.8%) | 15 (25.9%) | $\chi^2$ = 0.133 | 0.716 |
| Age | 54.38 ± 9.5 | 54.40 ± 9.9 | t= -0.010 | 0.992 |
| Weight | 82 (76-89) | 89.5 (76-96) | Z = -1.696 | 0.090 |
| Height | 166 (160-171) | 167 (162-172) | Z = -1.568 | 0.117 |
| BMI | 30.7 (27-32.5) | 31.6 (27-33.4) | Z = -0.995 | 0.320 |
| WC cm | 85 (81-91) | 87 (81-94) | Z = -0.643 | 0.520 |
| HC cm | 100 (92-101) | 100 (90-106) | Z = -0.089 | 0.929 |
| WHR | 0.89 (0.82-0.92) | 0.88 (0.82-0.94) | Z = -0.281 | 0.779 |
| TC mg/dL | 205.9 ± 52.7 | 199.2 ± 57.1 | t= 0.688 | 0.493 |
| LDL mg/dL | 129 (120-144) | 122 (112-149) | Z = -0.857 | 0.391 |
| HDL mg/dL | 39 (34-48) | 40.5 (32.1-48) | Z = -0.677 | 0.499 |
| TG mg/Dl | 112.5 (80-145) | 123 (89-179) | Z = -1.555 | 0.120 |
| RBG mg/dL | 86.7 ± 24.8 | 88.2 ± 19.9 | t= -0.368 | 0.714 |
| CRP mg/L | 152.5 (145-170) | 163 (150-178) | Z = -2.004 | **0.045** |
| AST IU/L | 29.6 (26-39) | 30.5 (27-42) | Z = -1.612 | 0.107 |
| ALT IU/L | 30 (26-33) | 32 (29-47) | Z = -2.465 | **0.014** |
| AST/ALT ratio | 1 (0.9-1.25) | 0.96 (0.9-1.07) | Z = -1.383 | 0.167 |
| Bilirubin mg/dL | 0.75 (0.7-0.8) | 0.8 (0.7-0.9) | Z = -2.926 | **0.003** |
| Albumin g/dL | 4 (4-4.2) | 4 (4-4.2) | Z = 0.318 | 0.751 |

This table shows a statistically significant higher proportion of NAFLD by US and statistically significant higher levels of CRP, ALT and serum total bilirubin in those with detected Firmicutes as compared to those with detected Bacteroidetes.

**Table (4):** Comparisons of lab tests in those with and without NAFLD:

| Parameter | Group | | Test of significance | |
|---|---|---|---|---|
| | NAFLD (n=19) | No NAFLD (n=182) | Z | P value |
| CRP mg/L | 200 (190-205) | 150 (145-166) | Z = -6.819 | **<0.0005** |
| AST IU/L | 46 (44.5-47) | 29 (25-34) | Z = -6.406 | **<0.0005** |
| ALT IU/L | 49 (48.5-49.5) | 29.6 (25-33) | Z = -7.064 | **<0.0005** |
| AST/ALT ratio | 0.94 (0.92-0.96) | 1 (0.88-1.21) | Z = -1.965 | **0.049** |
| Bilirubin mg/Dl | 1 (0.9-1) | 0.8 (0.7-0.8) | Z = -6.090 | **<0.0005** |

This table shows a statistically significant higher levels of CRP, AST, ALT and serum total bilirubin in those with NAFLD as compared to those without. It also shows a statistically significantly lower AST/ALT ratio in those with NAFLD as compared to those without.

**Table (5):** Predictors of the likelihood of microbiota detection:

| Predictor | B | SE | Wald | P value | OR (95% CI) |
|---|---|---|---|---|---|
| **BMI** | 0.166 | 0.045 | 13.941 | **<0.001** | 1.181 (1.082-1.289) |
| **CRP** | -0.003 | 0.004 | 0.802 | 0.371 | 0.997 (0.989-1.004) |
| **AST** | 0.004 | 0.009 | 0.192 | 0.662 | 1.004 (0.986-1.022) |
| **TC** | -0.005 | 0.003 | 4.165 | **0.041** | 0.995 (0.989-1.000) |

B: binomial logistic regression coeffient, SE: standared error, OR: odds ratio.

A binomial logistic regression was performed to ascertain the effects of BMI, CRP, AST and TC on the likelihood that participants have microbiota. The logistic regression model was statistically significant, $\chi^2$ (4) = 21.760, p<0.001. The model explained 13.9% (Nagelkerke $R^2$) of the variance in microbiota detection and correctly classified 71.6% of cases. Sensitivity was 87.9%, specificity was 45.5%, positive predictive value was 72.2% and negative predictive value was 70%. Of the four predictor variables only two were statistically significant: BMI (P<0.001), and TC (p=0.041) as shown in Table (5). Increasing BMI and decreasing TC were associated with an increased likelihood of exhibiting microbiota.

For BMI, one unit increase has 1.18 times higher odds that participate will exhibit microbiota detection.

For TC, for every one mg\dl decrease, there is 0.995 times higher odds that participate will exhibit microbiota detection.

**4. Discussion**

The prevalence of several chronic diseases are growing worldwide; of these, obesity is the primary culprit and has been major concern for decades [10]. Recent evidence suggests that the gut microbiota, affect nutrient acquisition and energy regulation; it further suggests that obese and lean people have different gut microbiota [11], [12]. In the past fifteen years, many scientists were exploring the association of the gut microbiota and some diseases, and the possibility for treatment affecting the gut microbiota. Conclusions from these studies resulted in inconsistent findings of the association between gut microbiota and obesity [13]. Firmicutes and Bacteroidetes are the dominant bacteria, accounting for approximately 99% of the whole microbiota [14], [15].

We conducted this study as a further step clarify the relationship between obesity and gut microbiota.

In this study, there was statistically significantly higher proportion of microbiota (Bacteroidetes / Firmicutes) in obese patients as compared to individuals with ideal weight and those who are overweight. Also presence of microbiota was associated with higher metabolic syndrome rate, CRP, AST.

In an elegant series of experiments, found that young conventionally reared mice have a 40% higher body fat content and 47% higher gonadal fat content than germ-free mice even though they consumed less food than their germ-free counterparts [16]. These investigators showed that the microbiota promoted absorption of monosaccharides from the gut and induced hepatic lipogenesis in the host, responses mediated by 2 signaling proteins, carbohydrate response element-binding protein (ChREBP) and liver sterol response element-binding protein type-1 (SREBP-1) [17]. Human studies have provided support for these findings. Treatment of humans with polymyxin B, an antibiotic that specifically targets gram-negative organisms, was shown to reduce lipopolysaccharide (LPS) expression and hepatic steatosis [18].

Another study reported that patients with type 2 diabetes had higher LPS levels than did a well-matched group of control participants without diabetes [19].

The gut microbiota composition and dysbiosis influence on the hormone orchestration and indirectly affect the appetite. This match with study reported how the intestinal infusion of *Escherichia coli* proteins in mice affects the increase of plasmatic levels of glucagon like peptide-1 (GLP-1) and peptide YY (PYY). They found that the changes in the gut microbiota (dysbiosis) induced changes in the plasmatic levels of GLP-1 and PYY, which are hormones that affect the hypothalamic arcuate nucleus and decrease appetite [20].

In study the association between some genus from the gut microbiota and the plasmatic levels of ghrelin and leptin. They noticed that there is a significant positive correlation between the number of Bifidobacterium and Lactobacillus and a significant negative correlation between the genus Clostridium, Bacteroides and Prevotella with the plasmatic levels of leptin. Also, they noticed that the plasmatic levels of ghrelin are negatively correlated with the genus Bifidobacterium, Lactobacillus and B. coccoides and positively correlated with Bacteroides and Prevotella [21].

Regarding the distribution of microbiota types according to the BMI categories in our study, there were no statistically significant difference between study groups, but we found that Firmicutes was associated with presence of NAFLD, higher CRP, ALT, and bilirubin levels.

Studies in animals fed high-fat or high-fiber diets showing higher Firmicutes and Bacteroidetes abundances, respectively [22]. In addition, a study was performed to report the differences in gut microbiota between obese and non-obese Japanese subjects. Obese subjects had significantly reduced numbers of *Bacteroidetes* and higher *Firmicutes* to *Bacteroidetes* ratios compared with non-obese subjects. The diversity of the bacteria was also significantly greater in the obese subjects than the non-obese subjects [23].

Other study serially monitored the fecal gut microbiota in 12 obese participants in a weight-loss program for a year. Members of the Bacteroidetes and Firmicutes divisions dominated the microbiota, and bacterial flora showed remarkable intraindividual stability over time. Before diet therapy, obese participants had fewer Bacteroidetes and more Firmicutes than lean control participants. After weight loss, the relative proportion of Bacteroidetes increased, while Firmicutes decreased, a finding that correlated with the percentage of lost weight and not with changes in dietary caloric content [15].

Similar findings were reported in children living in rural African areas, who consumed a traditional diet rich in fiber and showed higher proportions of Bacteroidetes and lower of Firmicutes, compared to children from western countries whose diet included large amounts of protein, fat, sugar, and starch [24].

Based on these results and others obtained from obese animals and humans, [13], [16], [25- 27] it has been proposed that the Firmicutes were more effective in extracting energy from food than Bacteroidetes, thus promoting a more efficient absorption of calories and the subsequent weight gain [28].

However, in opposition to these results, a number of studies did not observe any modifications of this parameter or even reported decreased Firmicutes/Bacteroidetes ratio in obese animals and humans [29- 33].

The fact that, in most of the studies, the obese patients showed lesser bacterial diversity than the lean subjects, suggests the existence of other compositional changes at family, genus, or species level, which might be more relevant than the Firmicutes/Bacteroidetes ratio [34], [35].

On the other hand, a few studies did not find any correlation between gut microbiota composition and variations in body weight. However, they produced intriguing data that suggest hypotheses about the specific

impact of several phyla or genera on obesity. Although some failed to detect a significant association between body mass index (BMI) or absolute weight loss and the relative concentration of populations of the major groups of human colonic bacteria, including Bacteroidetes, between obese and non-obese subjects [29].

Discrepancies between findings from different studies may be explained by different experimental setup involving sometimes effective life-style changes and sometimes not.

This study showed that the cases with detected microbiota had higher percentage of NAFLD as detected by US (11.3%) as compared with the cases with no detected microbiota, although this difference didn't reveal a statistically significant value.

The liver is constantly exposed to signals by the digestive system, gut microbes and their particles. Alterations in the gut microbiota composition are firmly connected with risk for disorders in the liver associated with obesity, such as non-alcoholic fatty liver disease (NAFLD). [36] in our study, a significant higher abundance of *Firmicutes* and a lower abundance of *Bacteroidetes* were exhibited in individuals with NAFLD compared with those without NAFLD, this in agreement with other study who found that Firmicutes are more frequent in NAFLD than non obese control group [38]. While some found that the NAFLD severity is associated with the degree of dysbiotic gut microbiota, especially with the number of *Bacteroidetes* related to NAFLD [39]. This finding was partly in agreement with the findings of who have demonstrated lower *Firmicutes* and higher *Bacteroidetes* abundances in obese patients compared with lean healthy controls [31].

To the best of our knowledge, this is the 1$^{st}$ study to find a binomial logistic regression was performed to ascertain the effects of BMI, CRP, AST and TC on the likelihood that participants have microbiota. Of the four predictor variables only two were statistically significant: BMI ($P<0.001$), and TC ($p=0.041$) as shown in Table (5). Increasing BMI and decreasing TC were associated with an increased likelihood of exhibiting microbiota. For BMI, one unit increase has 1.18 times higher odds that participate will exhibit microbiota detection. For TC, for every one mg/dl decrease, there is 0.995 times higher odds that participate will exhibit microbiota detection.

## 5. Conclusion

Gut microbiota and obesity releatioship is still inconclusive. This study suggest that diversity of gut microbiota has an important role in the regulation of body fat accumulation resulting in obesity and its complications including metabolic syndrome and fatty liver, this may be a promising treatment for obesity and preventing its dangerous sequelae.

Further studies are needed to confirm microbiota diversity as a cause of obesity.

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