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Research Article

Comparison of Gut Bacteria Structures Between Obese and Non-Obese Subjects in Libya

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Abstract

Background: Obesity has become main public health concerns globally. The main player in development of obesity that has been pointed out for two decades is the gut bacteria. Alteration in gut bacteria structure is associated with obesity development.

Aim, our study aimed to identify differences in gut bacteria structures between obese and non-obese subject. Besides, to determine whether there is correlation between fecal gut bacteria and anthropometric measurements, FSB, HbA1and lipid profile. Methods: the fecal microbiota of 61 Libyan volunteers was observed using conventional culture method.

Results: significant decreased was detected in prevalence of Prevotella copri (p. copri) in obese subject comparing to non- obese. The abundance of P. copri negatively correlated with age (r = -0.529, p = 0.001), BMI (r = -0.580, p = 0.0003), TC (r = -0.47, p = 0.001), LDL-C (r = -0.320, p = 0.05), and HDL-C (r = -0.409, p = 0.02) and positively correlated with WC (r = 0.501, p = 0.003).

Conclusion: P. copri was significantly less prevalent in obese individuals suggests its beneficial effects on BMI and lipid metabolism. This was confirmed by the negative correlation between P. copri and BMI, LDL, and HDL. The age-related decline in P. copri further exacerbates these metabolic disturbances. However, its positive correlation with WC may reflect a more nuanced relationship with fat distribution, particularly abdominal fat. This highlights the complexity of the gut microbiome's role in metabolic health and obesity, where different bacterial species can have distinct, sometimes opposing, effects on various metabolic parameters

Keywords: Obesity; Fecal Gut Bacteria; BMI; FBS; Lipid Profile

Introduction

Obesity is one of the common health problems in the world. According to the World Health Organization (WHO) facts, the prevalence of obesity increased more than doubled between 1990 and 2022. The main causes of obesity are modern lifestyle including physical inactivity and a high-caloric intake leading to an imbalance of energy intake and energy expenditure. Treating obesity and obesity-related conditions such as heart disease, diabetes, and cancer cost countries lots of money [1].

Recently, the relationship between obesity and Gut bacteria was also noticed. According to animal and human studies, alterations in the gut microbiota may be an important issue associated with obesity [2-4]. This imbalance in gut microbiota is categorized by a decrease in the number of gram-negative bacteria and increase of gram-positive bacteria [5]. For instance, obesity and impaired glucose metabolism were associated with an altered ratio between the two major phyla in the human gut, Firmicutes and Bacteroidetes [6-8]. Additional Studies indicated that obese people have a smaller range of gut microbiome than those with a moderate weight [9].

This change in the rate of Bacteroidetes and Firmicutes was also observed in individuals who lost weight as a result of gastric bypass surgery [10,11]. It is recognized that the consequences of gut dysbiosis can be increased energy uptake and intestinal permeability, and decreased microbial diversity [12]. According to these findings there is a distinct distribution of gut bacteria in obese individuals compared to those with a normal weight [13]. However, other elements such genetic background and diet can associated with gut microbe in obesity development [14].

Interestingly, modulation of gut bacteria positively has been used as an alternative choice to improve obesity and its complication. This modulation is taken several ways for instance, by using Probiotics or change life style to more heather approach and improvement habits food [12]. This leads to regulate and restore gut microbiota balance through promoting the growth/activity of useful bacteria and inhibiting those of harmful bacteria. However, to our knowledge, there has been no study conducted on gut alteration and obesity in Libya. Therefore, the target of this study was to determine whether there is difference in gut bacteria between non-obese and obese people in Libya. Also, to determine if there is association between some blood biochemistry and anthropometric factors with gut bacteria content.

Material and Method

Study population

Total of 61 participants, 30 female and 31 males were involved in this study. They divided into two groups based on BMI, 27 non-obese (BMI = 18-25.9), and 34 obese individuals (BMI ≥ 30). The average of BMI was 22.50 and 37.1 for participants, respectively. Demographics data about age, and gender were collected from participants by questionnaire. Weight and height measurements were used to calculate body mass index by usual formula, weight (kg)/height (m^2). Hip circumferences (HC) and waist circumferences (WC) were measured by tape for calculation waist-to-hip circumferences ratio (WHR) by dividing WC in cm by HC in cm. The agreement and sniggered for all participants including in this study were gotten after giving full informed consent and in accordance with the ethical principles of the Helsinki Declaration. Ethical approval was obtained from the university's research ethics committee.

Sample collection and Laboratory analysis

Blood samples were taken from all participants at 08:00 and 10:30 after fasting 10-12 hr. All biochemical parameters including fasting blood sugar (FBS), glycosylated hemoglobin (HbAc1 %), lipid profile such as total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol, (HDL-C) and triglycerides (TG) were analyzed in Razi laboratory by following the same technic methods as mentioned in our previous study in 2020 [15]. Also, fecal sample was collected for investigation bacteria type by culture-based methods. In that method, blood agar, nutrient agar, and agar diffusion methods were utilized to support microbial growth in samples obtained from both non-obese and obese patients. Inoculum suspension was applied to all surface isolated samples. The plates were initially stored at 4 (±2)°C for one hour and then incubated at 37 (±2)°C for 24 hours to promote bacterial growth. After incubation, microbial colonies were examined, and both the number and types of bacterial species present were identified.

Statistical analysis

SPSS version 20 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp) were used for analysis Data. Continuous data such as age, FSB, HbA1c, anthropometric measurements and lipid profile were assessed for normality using the Kolmogorov-Smirnov test. Continuous data were expressed as mean with standard deviation (SD) while categorical data related to gut bacteria abundances were presented as percentage. For comparison mean values of clinical data, the student *t* test was used whereas Chi-square was utilized to determine difference between two samples groups for relative abundances of gut bacteria. Spearman correlation coefficient was applied for determination relationship between gut bacteria contents in obese group and studied parameters. $P \leq 0.05$ was considered statistically different.

Result

This study conducted from 2022 to 2023 and involved 61 participants. Participants divided dependent on BMI into two groups, 27 non-obese (F/M: 12/15) with BMI (18-25.4) and 34 obese individuals (F/M: 18/16) with BMI ≥30 %. All the data of participants such as Age, gender, FSB, HbA1c, Lipid profile and anthropometric

Parameters	Non-obese (n = 27)	Obese (n = 34)	p-value
Gender (f/m)	12/15	18/16	
Age (year)	35.1 ± 13.7 (18-61)	32.3 ± 8.2 (39-70)	0.02
Weight (Kg)	64.6 ± 8.15	101.35 ± 24.22	0.001
BMI (kg/m^2)	22.50 ± 2.13	37.1 ± 5.71	0.001
WC (cm)	86.25 ± 9.01	103.7 ± 6.72	0.001
HIP (cm)	46.07 ± 7.9	61.52 ± 9.20	0.001
W/HR (%)	1. 91 ± 0.29	1.71 ± 0.24	0.09
FBS (mg/dl)	95.7 ± 12.7.62	100.5 ± 7.5	0.12
HbAc1	4.2.61 ± 1.9	5.4 ± 1.84	0.02
TC (mg/dl)	154.07 ± 36.1	169.44 ± 25.4	0.05
TG (mg/dl)	96.8 ± 52.03	100.50 ± 38.9	0.45
LDL- C (mg/dl)	87.66 ± 24.9	106.41 ± 21.23	0.002
HDL- C (mg/dl)	45.8 ± 5.3	42.35 ± 6.4	0.02
VLDL C - (mg/dl)	18.83 ± 10.1	19.9 ± 8.01	0.323

Table 1: Clinical characteristics of the participants.

Data are presented as the means and the STD (standard diffusion). F, female; M, male; BMI, Body Mass Index; WC, waist circumference, W/HR; waist to hip to Ratio; FBS, fasting blood Sugar; HbA1c, hemoglobinA1c; TC, total cholesterol; TG, Triglyceride; HDL-C, high density lipoprotein cholesterol; LDL-C, low density lipoprotein cholesterol; VLDL-C, very low density lipoprotein cholesterol.

measurements were described in table (1). The mean age of non-obese was (35.1 ± 13.7) ranging between 18 and 61 while for obese group, was (32.3 ± 8.2) ranging between 39 and 70. By using *t* test, significant difference was detected in some anthropometric measurements between two groups. Obese subjects had significantly higher weight, BMI, WC and Hip ($p < 0.01$) while no difference was seen in WHR (p, 0.09). Also, a significant increase was seen in levels of HbA1c, TC, LDL-C and HDL-C (p, 0.02, 0.05, 0.002, 0.02) whereas no significant difference in levels of FBS, TG and VLDL-C ($p > 0.05$). Conversely, a significant decrease was observed in level of HDL-C in obese group comparing to non-obese (p, 0.02). Related to fecal Gut bacteria and after using $\chi 2$ test, there was no a significant difference at level species in abundance of each *Bacteroides vulgatus, Bacteroides rodentium, Escherichia coli, staphylococcus aureus, Lactobacillus acidophilus* and *bucillus subtilis* between two groups ($p > 0.05$) as shown in figure (1). However, there was significantly lower in abundance of *Prevotella copri* (p. copri) in obese individuals compared to non-obese group (96.3% vs 67.6%, $p < 0.001$). Although, it was not significant, the highest relative abundance was seen in prevalence of *L. acidophilus* (96.3% vs 97.1%, $P > 0.05$) and followed by *S. ureses* (92.6% vs 92.6%, $P > 0.05$) and then *E. coli* (81.5% vs 91.2%, $P > 0.05$) in both non-obese and obese group respectively. Similarly, the relative abundance of both *B. vulgatus*, (81.5% vs 82.4%, $P > 0.05$) and *B. rodentium* (81.4% vs 82.3%, $P > 0.05$) was nearly equal in both groups. Conversely, the abundance of *B. subtilis* was the lowest species of bacteria in both groups and it was slightly higher in obese subjects comparing to non-obese subjects (63.0% vs 67.6%, $P > 0.05$).

Due to the significant difference in abundance of *P. copri* between two groups, the correlation examination was done by using Spearman correlation coefficient to determine which factors had important relationship with this type of bacteria. As shown in (Table 2), there was significant negative association between *P. copri* and age (r, -0.529; p, 0.001), BMI (r, -0.580; p, 0.0003), and WC (r, 0.501; p, 0.003), however; there was no association with body weight, Hip, FBS and HbA1c. Regarding to lipid profile, negative significant correlation was observed between *P. copri* and TC (r, -0.47; p, 0.001), LDL-C (r, -0.320; p, 0.05), and HDL- C (r, -0.409; p, 0.02) while no association was seen with TG and VLDL-C.

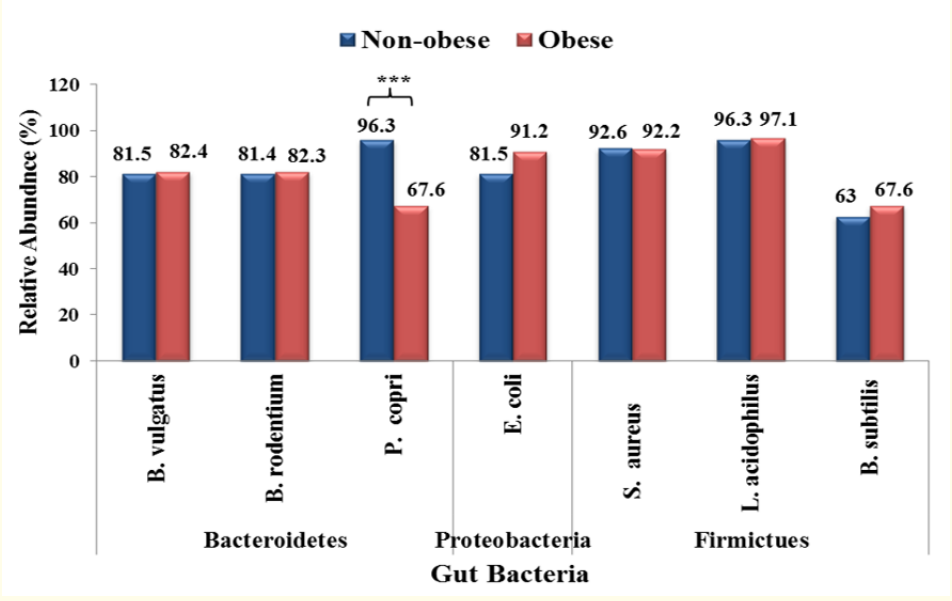

Figure 1: Relative abundance (%) of gut microbiota at species in non-obese and obese individual at level of species. Bars represent the reads percentage found by conventional culture method using the X2 test. *** = $p < 0.001$.

Table 2: Spearman correlation coefficient between *Prevotella copri* and study parameters.

Parameters	*Prevotella copri*	
	Correlation coefficient	p. values
Age	-0.529	0.001
Body weight	-0.310	0.07
BMI	-0.580	0.0003
WC	0.501	0.003
HIP	0.096	0.59
W/HR	0.202	0.252
FSB	-0.122	0.49
HbAc1	0.086	0.63
TC	-0.471	0.001
TG	-0.083	0.639
LDL	-0.320	0.05
HDL	-0.409	0.02
VLDL	-0.042	0.815
Data are presented as Spearman correlation coefficient (*P*-value).		

Discussion

This study examined gut bacteria at the species level in stool samples from 61 volunteers, divided into two groups based on BMI 34 obese and 27 non-obese individuals, using a culture method. The goal was to determine, first, whether there was a difference in bacterial content between obese and non-obese individuals, and, second, whether this difference was related to certain biochemical and physical factors related to metabolism and influencing obesity. Our results displayed that there was no difference in the proportion each of these bacteria *B. vulgatus*, *B. rodentium*, *E. coli*, *S. aureus*, *L. acidophilus* and *B. subtilis* between two study groups, except for a statistically significant difference was observed in the species of bacteria *P. copri*. The proportion of *P. copri* was statistically significant lower in obese patient comparing to non-obese group. In the present study, correlation ship analysis was also applied between *P. copri* and study parameters for obese group, it was found that this species of gut bacteria have a statistically significant and negative relationship with BMI, and age, but a positive relationship with WC. There was also a statistically significant and inverse relationship with levels of TC, LDL, and HDL in the same group. Notably, *P. copri* is an anaerobic, Gram-negative, non-spore-forming bacterium, often associated with a diet rich in fiber and plant-based foods. It is also known for its ability to ferment complex carbohydrates and dietary fibers, leading to the production of short-chain fatty acids (SCFAs) like acetate, propionate, and butyrate [16]. Non-obese individuals often have a higher intake of fiber-rich diets, which may support *P. copri* growth, whereas obese individuals tend to consume lower-fiber, high-fat diets that do not favor *P. copri* proliferation [17], therefore, this could explain high prevalence of *P. copri* in non-obese and its reduction in obese patient.

Regarding to the inverse relation of *P.copri* with age, gut microbiota composition knowledgeably shifts with age [18,19] often becoming less diverse and less rich in beneficial bacteria like *P. copri* [16]. In older obese individuals, the decline in *P. copri* may contribute to worsening metabolic parameters, such as increased LDL, cholesterol, and BMI.

The negative correlation between *P. copri* and BMI suggests that higher levels of this bacterium may be linked to a healthier body weight. This could be because *P. copri* helps improve gut health and promotes a favorable metabolic profile by producing SCFAs that play a role in appetite regulation and energy homeostasis [20]. For example, Propionate and butyrate can promote the release of satiety hormones like GLP-1 and PYY, which help regulate food intake and body weight [21]. Reduced *P. copri* in obese individuals might contribute to increased caloric intake and weight gain due to decreased satiety signals. Additionally, *P. copri* has been related to regulate gut microbiota composition, which is often associated with a healthier metabolic profile [20]. However, the positive correlation with WC suggests that *P. copri* has a possible role in central adiposity in despite of its apparent benefits on BMI in obese patients. This further complicating the interpretation of its role in obesity. These contrasting associations may reflect the complex relationship between gut microbiota and different measures of adiposity. While BMI represents overall body fat, WC is more specific to visceral fat, which is metabolically distinct and more closely associated with adverse health outcomes [22]. The differential associations may also indicate functional differences within *P. copri* strains or variations in host-microbe interactions across individuals [23-25]. Further suggesting explanation is that, the effect of *P. copri* may be masked by other confounding factors such as diet, genetics, or overall metabolic status [26-28]. Regarding to the lipid profile, *P. copri* correlated inversely with TC, LDL, and HDL levels proposes that *P. copri* may play a role in modulating cholesterol levels. The production of propionate by *P. copri* could potentially inhibit hepatic cholesterol synthesis, thereby promoting a reduction in LDL levels. One possible mechanism underlying this effect may involve the modulation of bile acid metabolism by *P. copri*, leading to increased bile acid excretion, which subsequently lowers circulating cholesterol levels [29]. In individuals with obesity, the reduced abundance of *P. copri* may result in diminished SCFA production, thereby contributing to elevated LDL and total cholesterol levels.

Collectively, the existence of a difference in the proportion of gut bacteria between two groups depend on BMI remains a subject of considerable debate in scientific literature. Some studies report such differences [8,30-32] while others do not [33,34] largely due to the variability and inconsistency of results across studies. Some research has found no significant differences [35] while others have identified variations at the phyla or species level, or both [6,7]. These inconsistencies may be attributed to several factors,

including study conditions such as sample size, ethnicity [36], dietary habits [33,37]. Furthermore, the degree of obesity is a critical variable, as evidenced by differences in the definition of obesity between studies [38]. For example, Japanese study defined obesity as a BMI greater than 25 [39] while a Chinese study used a BMI greater than 28 [40]. This variation in the criteria for obesity is an important factor influencing study outcomes, including in our current study, where the obesity threshold was set at BMI ≥ 30. Additionally, certain studies indicted to the vital role of gender and age factors in studies outcomes [41]. In our study, it was observed an inverse relationship between *P. copri* and age. The study sample size also plays a crucial role influencing the results. Our study is limited by both sample size and the culture-based methods, which may be considered less advanced compared to metagenomic analysis method and the real-time PCR assays that offer more comprehensive and detailed analyses. Despite these limitations, our findings indicate an inverse correlation between *P. copri* bacteria and both lipid metabolism and BMI, although a positive association with abdominal fat was observed. This suggests that *P. copri* may play a beneficial role in regulating metabolism and body mass index. However, the dual effects observed both positive and negative indicate the complexity of bacterial interactions, which could be influenced by factors such as metabolic hormones, sex [37,40,42] or interactions with other gut bacteria depend on host genetics and diet nature [9].

Conclusion

Our study revealed that *P. copri* was significantly less prevalent in obese individuals compared to non-obese individuals, suggest that *P. copri* plays a significant role in metabolic health, with its negative correlation to BMI, TC, LDL, and HDL indicating its potential beneficial effects on BMI and lipid metabolism. The positive correlation with WC suggests that *P. copri's* impact on fat distribution, particularly abdominal fat, is complex and demand further exploration. The higher prevalence of *P. copri* in lean individuals is likely influenced by dietary patterns, particularly high fiber intake, which promotes beneficial SCFA production, contributing to lower TC, LDL, and BMI. In contrast, the lower levels of *P. copri* in obese individuals may contribute to metabolic disadvantages, such as elevated cholesterol and increased BMI, due to reduced SCFA production and alterations in gut microbiota composition. Additionally, the age-related decline in *P. copri* further exacerbates these metabolic disturbances, highlighting the complexity of the gut microbiome's role in obesity and metabolic health. Therefore, more investigations are needed for determining functional roles of these bacteria which it could offer insights into innovative therapeutic strategies for treatment obesity and its associated metabolic disorders.

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