The Association of Gut Microbiota with Resting Metabolic Rate in Overweight/Obese Women: A Case-Control Study

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Abstract

Purpose

Low Resting Metabolic Rate (RMR), as a risk factor for obesity, can be affected by many factors. Indeed, genetic and environmental factors are variables taken into account when predicting RMR, and may contribute to a high inter-individual variance. Besides the well-known causes of obesity, researchers have demonstrated the contribution of gut microflora in obesity and energy expenditure. Therefore, the goal of the current study was to compare the *Firmicutes/Bacteroidetes* ratio and the relative abundance of, Prevotellaceae, Faecalibacterium prausnitzii, bifidobacterium spp, lactobacillus spp, Akkermansia muciniphila, Bacteroides fragilis, and Escherichia coli in two groups of people with normal and low RMR in overweight/obese women in Iran.

Results

The abundance of *F. prausnitzii* ($p > 0.001$), *B. fragilis* ($P = 0.02$), and *Firmicutes* phylum ($P = 0.02$) were significantly higher in the controls compared to the cases, and showed significant positive association with RMR, ($\beta = 1.29 \times 10^{-5}$, $P = 0.01$), ($\beta = 4.13 \times 10^{-6}$, $p = 0.04$), and ($\beta = 7.76 \times 10^{-1}$, $p = 0.01$), respectively. Regarding *Lactobacillus*, the results showed a significant positive association with RMR ($\beta = 1.73 \times 10^{-4}$, $p = 0.01$).

Conclusion

Intestinal microbiota may be associated with host metabolism. Therefore, future work should investigate, using clinical trials, the impact of manipulating gut microflora to positively influence energy expenditure.

Background

Obesity, as a worldwide epidemic, continues to be increasing in prevalence in developed and developing countries [1–3]. As such, it is the fifth greatest cause of non-communicable diseases [4]. Currently, over 1 billion people around the world are overweight or obese [5], whilst, in Iran, the age-adjusted prevalence of overweight /obesity is estimated to be 42.8% and 57% in men and women, respectively [6]. Obesity is a complex and multifactorial disorder that is generally caused by chronic positive energy balance when energy intake (EI) surpasses energy expenditure (EE). Daily energy expenditure is composed of three major components: resting metabolic rate (RMR) (60 - 80%), activity-related energy expenditure (AEE) (10 - 30%), and diet-induced thermogenesis (DIT) (10%) [7, 8]. Accordingly, RMR (the largest component of EE) is the energy requirement to maintain all vital body functions without any physical activity or additional energy expenditure [9]. Moreover, according to previous studies, low RMR is known as a potential risk factor for weight gain [10, 11]. However, there is an inter-individual variation of RMR, mostly caused by fat free mass [12–16] as well as age, fat mass, and sex [13, 17]. In general, women in comparison to men [18–22], and older adults compared to young adults [20, 23, 24], have lower RMR, which can be explained by differences in their muscle mass [25, 26]. Moreover, genetics [27], insulin level, blood pressure [23], and thyroid hormones are other factors affecting RMR [23, 28, 29]. Nevertheless, there is some other unexplained variation of RMR [13, 17]. Presently, due to many etiological factors, obesity is difficult to regulate and intestinal microbiota has been posited as a new factor affecting obesity and host energy metabolism [30]. Indeed, Bäckhed et al. [31] initially suggested this association, and several further studies have highlighted the

role of gut microbiota as a crucial factor affecting energy metabolism [32, 33], whilst some others have assessed the gut microbiota composition of obese and normal-weight subjects [34–37]. Regarding energy expenditure, in 2013, P. KOCEŁAK et al. [38] evaluated the association between gut microbiota and REE, where higher bacterial load was found in obese compared to normal weight individuals, but no independent association was seen with REE. In contrast, several animal studies have found that some bacteria, such as *Akkermansia muciniphila* [39], *Lactobacillus reuteri* [40] and *Lactobacillus gasseri* [41] could increase energy expenditure. However, the role of gut microbiota in the context of energy homeostasis, especially RMR, has not been clearly demonstrated in humans so far. Therefore, we sought to compare the abundance of some bacteria in overweight/obese women with normal and low RMR. To the best of our knowledge, this study would be the first in this context. Moreover, we also aimed to determine the association between the abundance of such bacteria and RMR among our studied population.

Methods

Study population

In the current matched case-control study, we enrolled 36 healthy women (18 cases and 18 controls), who had been referred to a nutritional laboratory according a public invitation in Tehran. We used a Telegram bot and a total of 1300 participants were signed up among which, 122 individuals were eligible samples. Finally, after assessing their medical history, 85 volunteers were qualified for the study (Figure-1). After a follow-up period of 2-3 months, to ensure no medication and supplementation, as well as no fluctuations in weight and other inclusion criteria were maintained, individuals were referred to the Nutrition Laboratory of the Faculty of Nutrition and Dietetics located at the School of Health of Tehran University of Medical Sciences for further evaluation. With the purpose of random distribution, the participants in the case and control groups were divided into paired blocks in terms of age, BMI, and RMR level. Each sample in the case group (low RMR) was randomly matched with a sample in the control group (normal RMR) with a maximum difference in age ± 2 years and BMI ± 2 units. Volunteers were randomly selected for the study based on the following inclusion criteria (for both cases and controls): $25 \leq \text{BMI} < 40$ (obesity and overweight), aged 18-50 years old. To ensure comparable data, we included the following exclusion criteria: use of antibiotics (within the previous 3 months) [79], use of alcohol, smoking, significant infection, history of diabetes, coronary, thyroid diseases or other hormonal disease and cancer. Use of medications or treatments effective on their RMR, use of supplementary vitamins and minerals, being pregnant, lactating or menopausal, daily or irregular intake of probiotics within the previous 2 months, history of digestive diseases, such as inflammatory bowel disease, irritable and constipation. Also those with gastrointestinal surgery, use of dietary supplements for weight loss during the past 6 months [38] were excluded. The inclusion and exclusion criteria of the samples were based on the individual's medical history or their own statements. The study protocol was approved by the Ethics Commission of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1398.562) and prior to the study, all subjects signed a written informed consent.

Demographic questions

Demographic questions were used to collect data about characteristics such as age, smoking status, education level, lifestyle, marital status, menopause, medical history, taking medication, supplement use etc.

Anthropometric assessment

For each participant, body composition, including weight, BMI, body fat mass (BFM), fat free mass (FFM), body fat percentage (%), waist to hip ratio and waist circumference were measured. All measurements performed by using a multi-frequency bioelectrical impedance analyzer, InBody 770 scanner (Inbody Co., Seoul, Korea). For all participants, a very low, safe electrical signal was sent from four metal electrodes through both hands and feet. The electrical signal passes quickly through water that is present in hydrated muscle tissue but meets resistance when it hits fat tissue. This resistance, known as impedance, can be measured to infer the proportion of fat free mass and fat mass. Measurement was conducted according to the manufacturer's guidance.

Resting Metabolic Rate measurements

RMR was measured by indirect calorimetry (MetaLyzer®3B, made in Germany). The RMR was assessed in the morning after a requested overnight fast (10-12 hour). It was measured under "resting conditions," which included no prior severe exercise, and abstinence from alcohol and caffeine. The indirect calorimetry device was calibrated before each assessment. To measure RMR (m-RMR), after 20 minutes of rest, the patients assumed the supine position without movement for 30 min, and the middle 20 min was used for calculation (the first and the last 5 min were ignored). We stratified participants based on measured RMR (m-RMR) and predicted RMR values (p-RMR) [80]. Patients were defined as "hypometabolic" when their measured RMR was less than 85 % of the predicted RMR, based on the Harris and Benedict equation [81], or "normometabolic" when it was within $\pm 15\%$ of the predicted RMR. During the luteal phase of the menstrual cycle, RMR typically rises and is lower during menstruation. For this purpose, indirect calorimetry performed for all premenopausal women during the follicle process [82-84].

Dietary assessments

Dietary intake was assessed by a semi-quantitative 147-item food frequency questionnaire (FFQ) that has been validated in previous work [85]. Questionnaires were completed in the presence of a trained nutritionist, and participants reported the intake frequency of each food item over the past year. Household measurements and servings were then converted into weight (grams per day). Dietary intakes were analyzed using NUTRITIONIST 4 (First Data Bank, San Bruno, CA) software for estimating energy and nutrient intake.

Physical activity assessment

For evaluating physical activity in the form of metabolic equivalent hours per week (MET-h/wk), the short form of the International Physical Activity Questionnaire (IPAQ) was used, whose Persian language version has been validated by Moghaddam et al. [86]. Scores were calculated according to the frequency and time spent on light, moderate, high, and very high-intensity activities, based on a list of common daily activities.

Blood sampling

Following a 12-hour overnight fasting, 10 cc of venous blood sample was drawn between 7 and 10:30 a.m., and immediately divided. Half of each sample was kept for 30 minutes at room temperature until clotting, then, blood samples were centrifuged at 3000 g for 20 minutes, and decanted into several separate clean micro-tubes and stored in a freezer at -80°C for further analysis.

Biochemical and hormonal assessments

All hormones were determined by using enzyme-linked immunosorbent assay (ELISA) method. Leptin, ghrelin and insulin levels were assessed using a LDN kit (Nordhorn, Germany) with a sensitivity of 0.50 ng/ml, a Crystal Day Christian Day kit with a sensitivity of 0.01 ng/ml, and an IBT kit (infinitum biotech, IBT; Netherland) with a sensitivity of 0.11 μ U / ml. Intra- and inter-assay coefficients of variation (CV) reported by the manufacturer for leptin, ghrelin, and insulin were 3.7– 5% and 5.9–5.8%, CV<8% and CV<10%, and 3.7-4.2%, and 3.7-4.2%, respectively.

Fecal sampling and DNA extraction

Participants were asked to collect their stool samples in a conventional laboratory plastic container dedicated to fecal sampling. The samples were moved immediately to the laboratory in ice packs and stored at -80 °C (flash frozen) upon arrival (within 2 h.), before further processing. Extraction of total bacterial DNA from 200 mg of each stool sample has been done using QIAamp The Fast DNA Stool Mini Kit (51604) (Qiagen, Hilden, Germany) was used according to manufacturer’s instructions. The purity and concentration of the extracted DNA were determined by Nanodrop spectrophotometer (Thermo Scientific NanoDrop, USA) [87]. The extracted DNAs were stored at -20 °C until further analysis. By using the nucleotide BLAST in NCBI, the specificity of the primers was evaluated. The specific sequences of primers are shown in Table 1.

q-PCR analyses

The abundance of bacteria was analyzed using Quantitative real-time PCR based on SYBER green method (LightCycler® 96 SW 1.1; Roche, Germany) [35,87,88]. Each 10 μ l of q-PCR reaction was composed of SYBR Premix Ex Taq II (RR820L; Takara, Japan), 0.5 μ l of each of the specific 16s rRNA primers [35,87-94] (Table 1), and 1 μ l of the DNA template. The q-PCR reactions were carried out in duplicate using LightCycler® 8-Tube Strips (clear; Roche). An appropriate annealing temperature was used for designing the amplification program: 1 cycle of 95 °C for 1 min, 40 cycles of denaturation at 95 °C for 5 s, then annealing at 55 °C for 30 s, and extension at 72 °C for 30 s. Finally, melting curve analysis was performed after amplification to confirm the specificity of PCR reactions, followed by 1 cycle at 95 °C for 5 s, 60 °C for 1 min, and 95 °C for 1 s.

Table 1. Primers used in this study

Target Bacteria	Forward (5' to 3')	Reverse (5' to 3')	Reference
Prevotellaceae	CACCAAGGCGACGATCA	GGATAACGCCYGGACCT	(89)
Faecalibacterium prausnitzii	GGAGGAAGAAGGTCTTCGG	AATTCCGCCTACCTCTGCACT	(88)
Bifidobacterium spp	TCGCGTCYGGTGTGAAAG	CCACATCCAGCRTCCAC	(92)
Lactobacillus spp	AGCAGTAGGGAATCTTCCA	CACCGCTACACATGGAG	(91)
Akkermansia muciniphila	CAGCACGTGAAGGTGGGGAC	CCTTGCGGTTGGCTTCAGAT	(87)
Bacteroides fragilis	CTGAACCAGCCAAGTAGCG	CCGCAAACCTTTCACAACTGACTTA	(93)
Firmicutes	GGAGYATGTGGTTTAATTCGAAGCA	AGCTGACGACAACCATGCAC	(94)
Bacteroidetes	AAACTCAAAGAATTGACGG	GGTAAGGTTCTCGCGCTAT	(35)
Escherichia coli	CATTGACGTTACCCGCAGAAGAAGC	CTCTACGAGACTCAAGCTTGC	(90)

Standard curve

For calculating the abundance of bacteria in each sample we made a comparison between obtained cycle threshold (CT) values with the standard curves using serial dilutions of DNA obtained from the standard strain *Escherichia coli* (ATCC 25922), with known DNA concentration. This curve enables measurement of each bacterium's DNA concentration from stool samples. As a semi-log regression line plot of CT value vs. log of DNA concentration, the standard curve is graphically represented.

Statistical analysis

The normality of the data was assessed using Kolmogorov-Smirnov test and was shown to be non-normally distributed. Categorical variables were presented as percentages, and continuous variables presented as median and interquartile range. Wilcoxon test and Chi-square or Fischer exact tests were used for comparison quantitative and qualitative variables, respectively, across the two studied groups. To assess associations between gut microbiota and RMR, linear regression was used. In an adjusted model, body fat percent was adjusted. All statistical analyses were performed using SPSS software (version 23, SPSS Inc., Chicago, IL, USA), whilst $P < 0.05$ was, *a priori*, considered statistically significant.

Results

We identified 18 individuals with low RMR (median age of 34 years; and median BMI of 28.65) and enrolled 18 participants with normal RMR as controls (median age of 35.5 years; and median BMI of 28.55) (age and BMI were matched with cases). Participant characteristics are presented in (Table-2). The median of measured RMR was significantly higher in controls compared to cases, (median: 1511.5, IQR: 163.25 vs median: 1303.5, IQR: 291) ($P < 0.001$). The median of age ($P = 0.28$), weight ($P = 0.40$), BMI ($P = 0.11$), and fat free mass ($P = 0.06$) were not significantly different among case and control groups. However, body fat percent was significantly different among groups, where controls had lower body fat percent (median: 40.35, IQR: 7.1) in comparison to the other

group (median: 44.4, IQR: 6.27.1) ($P= 0.03$). In addition, there was no difference in the median of leptin, insulin, and ghrelin levels between two groups ($P= 0.83$), ($P= 0.31$), ($P= 0.30$) respectively. In terms of qualitative variables, there were no significant differences in physical activity levels of cases and controls ($P= 0.87$). Moreover, there was no difference between energy intake and dietary intake of micro/macro nutrients and food groups among cases and controls (presented in Table-3) ($P> 0.05$).

Table 2
General characteristics of participants among cases and controls.

General characteristics of participants among cases and controls.			
Variable	Controls	Cases	P-Value ^a
	N=18	N=18	
Quantitative Variables			
Age (year)	35.5 ± 10.25	34 ± 7	0.28
Height (cm)	163 ± 6	158 ± 7.25	0.07
Weight (kg)	77.25 ± 13.6	74.6 ± 14.33	0.40
Body mass index (kg/m ²)	28.55 ± 5.48	28.65 ± 4.33	0.11
m-RMR ^b (kcal)	1511.5 ± 163.25	1303.5 ± 291	<0.001
RMR/kg ^c	20.11 ± 2.68	16.85 ± 2.39	<0.001
Fat free mass (kg)	46.8 ± 5.9	43.4 ± 9.1	0.06
Body fat mass (Kg)	32.6 ± 11.6	33 ± 9.5	0.18
Body fat percent	40.35 ± 7.1	44.4 ± 6.2	0.03
Waist to hip Ratio	0.93 ± 0.1	0.92 ± 0.06	0.45
Waist circumference (cm)	96.85 ± 16.13	93.65 ± 13.83	0.65
Systolic blood pressure	98 ± 21	98.5 ± 30.5	0.45
Diastolic blood pressure	67.5 ± 16	73 ± 14.25	0.06
Leptin	20.57 ± 5.65	20.40 ± 3.74	0.83
Insulin	14.81 ± 6.53	14.05 ± 6.37	0.31
Ghrelin	1.83 ± 2.47	2.18 ± 5.69	0.30
Qualitative Variables			
Physical activity			
less or never	10(55.6)	13(72.2)	0.87
Moderate	8(44.4)	3(16.7)	
High	0	2(11.1)	
* Data are presented as median ± interquartile range or number (percent).			
^a P-values are from Wilcoxon test for quantitative and Fischer exact tests for qualitative variables comparisons between two groups			
^b Measured resting metabolic rate, ^c Resting metabolic rate/weight (kg)			

P value <0.05 was considered statistically significant

Table 3
Dietary intake of micro/macro nutrients and food groups among cases and controls.

Variables	Controls, N=18	Cases, N=18	P-Value*
	Median $\pm$ IQR**	Median $\pm$ IQR	
Energy (Kcal)	2102.87 $\pm$ 591.38	2261.31 $\pm$ 1020.56	0.18
Protein(g/day)	77.13 $\pm$ 33.56	88.64 $\pm$ 42.61	0.50
Carbohydrate (g/day)	287.46 $\pm$ 80.52	320.1 $\pm$ 114.56	0.23
Fat(g/day)	71.37 $\pm$ 24.6	75.76 $\pm$ 36.9	0.56
Vitamin A(RAE/day)	586.08 $\pm$ 404.11	770.39 $\pm$ 485.84	0.06
Vitamin D(μ g/day)	1.38 $\pm$ 1.81	1.93 $\pm$ 1.73	0.37
Vitamin K(μ g /day)	285.9 $\pm$ 291.52	348.54 $\pm$ 453.4	0.47
Vitamin E(mg/day)	10.46 $\pm$ 3.38	9.27 $\pm$ 5.05	0.45
Vitamin C(mg/day)	133.08 $\pm$ 102.17	118.06 $\pm$ 83.45	0.88
Iron(mg/day)	28.71 $\pm$ 15.86	33.87 $\pm$ 34.85	0.17
Folate (mg/day)	495.73 $\pm$ 190.6	562.06 $\pm$ 162.7	0.33
Fiber(g/day)	34.35 $\pm$ 17.51	34.13 $\pm$ 22.88	0.16
Grains (g/day)	318.05 $\pm$ 112.48	345.35 $\pm$ 126.08	0.18
Fruit (g/day)	322.95 $\pm$ 223.2	269.7 $\pm$ 371.8	0.37
Vegetables (g/day)	249 $\pm$ 236.2	323.3 $\pm$ 203.95	0.18
Beans (g/day)	33.7 $\pm$ 46.58	39.85 $\pm$ 64.4	0.68
Diary (g/day)	294.45 $\pm$ 191.43	328.95 $\pm$ 319.08	0.31
Red meat (g/day)	21.2 $\pm$ 30.2	16.4 $\pm$ 17.23	0.21
White meat (g/day)	46.4 $\pm$ 48.33	38.7 $\pm$ 26.4	0.56
* Resulted from Wilcoxon test			
** Data are presented as median $\pm$ interquartile range			

P value <0.05 was considered statistically significant

In Table-4, median abundance of studied bacteria and phyla are presented. The results showed that bacterial load of *F. prausnitzii* (P<0.001) and *B. fragilis* (P= 0.02) in fecal samples of control groups who had normal RMR were significantly higher compared to those with low RMR in case groups. Furthermore, *Firmicutes* abundance increased was significantly in control groups (P= 0.02). No significant difference was found in the level of *Akkermansia*, *Lactobacillus*, *Bifidobacterium*, *Escherichia coli*, *Prevotella*, and *Bacteroidetes* phylum between the two groups (P > 0.05).

Table 4
Comparison of abundance of bacteria between cases and controls*.

Variables	Cases		Controls		P-Value**
	N=18		N=18		
	Median	Interquartile Range	Median	Interquartile Range	
Bacteria					
Akkermansia muciniphil CFU ^a /gr (X10 ³)	13194.79	236804.38	15536.07	394800.43	0.35
Bacteroides fragilis CFU/gr (X10 ⁵)	7464676.77	12736868.07	16996096.41	28344748.78	0.02
Bifidobactrium CFU/gr (X10 ⁴)	1030250.00	1047720.63	1455799.41	2726496.85	0.08
Escherichia Coli CFU/gr (X10 ⁴)	192403.18	1148809.48	210996.67	317666.96	0.71
Faecalibacterium CFU/gr (X10 ⁵)	5340303.00	4317291.18	9682001.42	10643646.47	<0.001
lactobacillus CFU/gr (X10 ³)	21286.19	75833.80	21723.46	40607.88	0.95
Prevotella CFU/gr (X10 ⁵)	725377.63	23939856.19	282798.00	19144690.17	0.56
Phylum					
Fir/Bac ^b	1178.15	2615.63	1926.81	1725.07	0.84
Baceriodetes	0.07	0.10	0.10	0.05	0.23
Firmicutes	84.12	131.82	197.08	253.73	0.02
*Data are presented as median ± interquartile range					
^a Colony forming unit					
^b Firmicutes/Baceriodetes					
** Wilcoxon test was used					

P value <0.05 was considered statistically significant

In order to discern the association between the bacterial abundance and RMR, we conducted linear regression, in crude and adjusted models. Our crude results manifested a positive association between *F. prausnitzii* ($\beta = 1.29 \times 10^{-5}$, $SE = 5 \times 10^{-6}$, $P = 0.01$), *B. fragilis* ($\beta = 4 \times 10^{-6}$, $SE = 2 \times 10^{-6}$, $P = 0.03$), *Lactobacillus* ($\beta = 1.61 \times 10^{-4}$, $SE = 5.5 \times 10^{-5}$, $P = 0.01$), and *Firmicutes* ($\beta = 7.52 \times 10^{-1}$, $SE = 2.74 \times 10^{-1}$, $P = 0.01$) with RMR (Table-5). Further, after adjustment for body fat percent as a confounder (because it was significantly different between groups), the above-mentioned associations remained statistically significant ($P < 0.05$).

Table 5
The association between resting metabolic rate and gut microbiota.

Variables		β -coefficient	Standard Error	P-Value*
Akkermansia muciniphila	Crude ^a	3.97×10^{-5}	9.1×10^{-5}	0.67
	Model 1 ^b	3.85×10^{-5}	9.3×10^{-5}	0.68
Bacteroides fragilis	Crude	4×10^{-6}	2×10^{-6}	0.03
	Model 1	4.13×10^{-6}	2×10^{-6}	0.04
Bifidobacterium spp	Crude	2.44×10^{-5}	1.3×10^{-5}	0.06
	Model 1	2.44×10^{-5}	1.3×10^{-5}	0.07
Escherichia coli	Crude	-2.43×10^{-5}	4×10^{-5}	0.55
	Model 1	-2.42×10^{-5}	4.1×10^{-5}	0.56
Faecalibacterium prausnitzii	Crude	1.29×10^{-5}	5×10^{-6}	0.01
	Model 1	1.29×10^{-5}	5×10^{-6}	0.01
Lactobacillus spp	Crude	1.61×10^{-4}	5.5×10^{-5}	0.01
	Model 1	1.73×10^{-4}	5.7×10^{-5}	0.01
Prevotellaceae	Crude	1.19×10^{-6}	1×10^{-6}	0.40
	Model 1	1.22×10^{-6}	1×10^{-6}	0.40
Fir/Bac ^c	Crude	-1.62×10^{-2}	1.77×10^{-2}	0.37
	Model 1	-1.61×10^{-2}	1.8×10^{-2}	0.38
Bacteroidetes	Crude	1.16×10^3	6.81×10^2	0.10
	Model 1	1.16×10^3	6.92×10^2	0.10
Firmicutes	Crude	7.52×10^{-1}	2.74×10^{-1}	0.01
	Model 1	7.76×10^{-1}	2.8×10^{-1}	0.01
* Calculated using linear regression				

a Not adjusted

b Model 1 is adjusted for body fat percent

c Firmicutes/Bacteroidetes

Discussion

The finding of the current study shows that the abundance of *B. fragilis*, *F. prausnitzii*, and *Firmicutes* were higher in those with normal RMR compared to those with low RMR. Moreover we found that with an increasing abundance of *B. fragilis*, *F. prausnitzii*, *Lactobacillus*, and *Firmicutes*, RMR concurrently increases. In order to discern the relationship between gut microbiota and obesity, numerous animal and human-based research [30, 35, 42–44] has been conducted [45]. Accordingly, studies have shown that energy homeostasis imbalances, low-grade inflammation, and insulin resistance result from dysbiosis, which can contribute to negative host metabolism regulation [46]. Moreover, since the first paper on the role of intestinal microbiota in host metabolism, including body weight regulation [31], the gut microbiome has been shown to have important functions, such as impacting on dietary energy harvesting and the control of anti-inflammatory and metabolism; indeed, any alteration in the composition of the gut microbiota may trigger and develop obesity, or vice versa [46, 47]. The rise in *Firmicutes* and decrease in *Bacteroidetes* concentrations (increase in F/B ratio) in obese vs. normal subjects has been shown in various animal and human studies [30, 48–50]. In the current study, there was no association between F/B ratio and RMR, which is concordant with the results of the previous study conducted on both normal and obese individuals [38]. However, we observed higher abundance of *Firmicutes* phylum in control groups (with normal RMR) and a positive association between this phylum and RMR. Dominancy of *Firmicutes* in people with normal RMR could be explained by some genus belonging to this phylum, such as *Lactobacillus spp.* and *Faecalibacterium prausnitzii*. In fact, in this study we observed significant positive correlation between *Lactobacillus spp.* and *Faecalibacterium prausnitzii* with RMR. Moreover, *Bacteroides fragilis* showed a positive correlation with RMR as well. There is a dearth of studies that have assessed the correlation between gut microbiota and RMR. Nevertheless, *F. prausnitzii* has been shown to be a butyrate producer that exhibits anti-inflammatory and protective effects against obesity [51]. Previous studies have demonstrated that consumption of fermentable carbohydrates could influence production of butyrate [52, 53]. Indeed, diets high in non-digestible carbohydrates can promote the abundance of some butyrate-producing bacteria, and thus contribute to higher levels of butyrate in plasma. Moreover, in animal studies, it has been shown that high circulation of butyrate in plasma may improve insulin sensitivity and increase energy expenditure [54, 55]. In addition, it has been proved that obese participants have reduced levels of plasma butyrate [56]. Intestinal gluconeogenesis, via the intestine-brain circuit, is activated by butyrate and propionate, thus promoting glucose regulation and metabolic benefits expressed by body weight [57]. The other mechanism by which butyrate can increase energy expenditure is via its capacity to promote thermogenesis and fatty acid oxidation by activation of 5'-AMP-activated protein kinase (AMPK), phosphorylation of peroxisome proliferator-activated receptor-gamma coactivator-1 α (PGC1 α) in muscle and liver, and up regulation of PGC-1 α and mitochondrial uncoupling protein-1 (UCP-1) in brown adipose tissues (BAT) [58, 59]. AMPK can inhibit acetyl CoA carboxylase via phosphorylation, and consequently reduces malonyl CoA synthesis [59]. With regard to *Lactobacillus spp.*, it seems that empirical results are equivocal; where some studies support its anti-obesity roles [60] while some others assert that it is obesogenic [61, 62] by considering its different genus [63]. Although, in the current study, there was no significant difference of abundance of *Lactobacillus spp.* between cases and controls, a significant correlation was found between their

frequency and RMR. In fact, our study is concordant with some animal studies [40] showing that some bacteria belonging to this family can promote energy expenditure. This study verified that *L. reuteri* 263 may have an impact on increasing energy expenditure by the up-regulation of Uncoupling protein-1 (Ucp1), presented in white adipose tissue (WAT), by a process called browning. The browning remodels the energy metabolism of WAT and converts it to a beige adipose tissue with higher energy consumption and greater thermogenesis and respiratory rate [64–66]. In other words, browning of WAT is a proposed mechanism to increase energy expenditure and decrease obesity. Moreover, Bungo Shirouchi et al. [41] asserted that *Lactobacillus gasseri* SBT2055 (LG2055) can elicit anti-obesity effects via increases in carbohydrate oxidation and contribute to the increase of energy expenditure. Indeed, the increase in butyrate production by LG2055 can activate G protein-coupled receptor41 (GPR41) and increase energy expenditure following enhancement of glucose tolerance. Indeed, research suggests that in the host, GPRs serve as receptors for free fatty acids (FFAs), among which, free fatty acid receptor 3 (FFA3/GPR41) can be activated via SCFAs, such as propionate, butyrate, and acetate [67–70]. Interestingly, animal studies have illustrated that GPR41 knockout mice have decreased energy expenditure and less glucose tolerance [71]; suggesting that short chain fatty acids (SCFAs) are able to increase the sympathetic nervous activity by GPR41, and consequently increase energy expenditure in animal models [72]. As we observed significant difference between *Bacteroides fragilis* abundance in both cases and controls with higher RMR, it seems that, in contrast to previous studies proposing obesogenic role of *B. fragilis* [73, 74], it may impose protective effects against obesity by improving RMR. As we discussed earlier, butyrate can increase energy expenditure via aforementioned mechanisms, and *B. fragilis*, that promotes mucin production, is a propionate producer, which is a precursor of butyrate [75]. In this way, a previous study found that *B. fragilis*, in combination with *Bifidobacterium infantis*, *Eubacterium limosum*, and whole leaf extract of Aloe vera, can increase production of butyrate [76]. Overall, findings have demonstrated that SCFAs can mediate the beneficial effects of prebiotics, and based on the individual microbiota, prebiotics can produce propionate or butyrate [77]. However, there is not enough human evidence to show the relationship between gut microbiota and host resting metabolic rate in overweight/obese individuals with low or normal RMR rate, and this study, for the first time, elucidates this issue. Since the composition of gut microbiota can differ based on different environmental factors, such as race, dietary intake, lifestyle, population-specific variations, and geographical area, it is necessary to determine gut microbiota pattern of each targeted population to manage obesity using proper strategies [78].

Conclusions

In conclusion, the results of this study showed, for the first time, that despite the absence of significant differences in abundance of, *A. muciniphila*, *Bifidobacterium*, *Prevotella*, and *E. coli* between subjects with normal RMR and low RMR, the abundance of *F. prausnitzii*, *B. fragilis* and *Firmicutes* phylum were significantly higher in fecal samples of individuals with normal RMR. In addition, based on the correlation of studied bacteria and RMR, our findings showed a positive association between *F. prausnitzii*, *B. fragilis* and *Firmicutes* phylum, as well as *Lactobacillus*, with RMR. Finally, by determining the gut microbiota effectiveness on host energy expenditure, proper strategies can be designed to manage obesity based on each targeted population. The main strength of the current study is that, for the first time, the relationship between gut microbiota and resting metabolic rate in Iranian overweight /obese women by matching two important confounders, age, and BMI, has been investigated. Moreover, we assessed dietary intake, body composition, leptin, ghrelin, and insulin levels, and physical activity of all participants, allowing a comprehensive profile of all participants. Nevertheless, despite the strengths noted above, there are some limitations of the present study. Regarding the case-control design of this study, we are not

able to discern any cause and effect association between gut microbiota and energy expenditure, and further prospective longitudinal studies and clinical trials are needed. Although we matched cases and controls by age and BMI, there are likely some other confounders which might affect our findings.

Declarations

Ethics approval and consent to participate

The study protocol was approved by the Ethics Commission of Tehran University of Medical Sciences (IR.TUMS.VCR.REC.1398.562). Then, written informed consent was obtained from all patients. All methods were performed in accordance with the declaration of Helsinki.

Consent for publication

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

KHM and SDS supervised the study; SM and SH collected the data; SM, SAB and AM did experimental analysis; SM and MSY analyzed the data; SM wrote the first draft with contributions from the other authors and CCTC, revised the manuscript. All authors reviewed and commented on subsequent drafts of the manuscript.

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Figures

Figure 1. Flowchart of sampling

Figure 1

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