Comparison of Gut Microbiota and Serum Biomarkers in Obese Patients Diagnosed with Diabetes and Hypothyroid Disorder

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Research

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Abstract

Background

Variations of serum biomarkers and bacterial diversity of the gastrointestinal tract in obese patients with diabetes or hypothyroid are poorly understood. This study broadened our understanding of recent findings in this regard.

Methods

A total of 120 obese patients (18 with diabetes, 23 with hypothyroid, and 79 patients without either diabetes or hypothyroid (control)) were recruited in this study. Serum biomarkers such as biochemical, hormonal (insulin and glucagon), and cytokine levels (IL-6, IL-1 β , TNF- α , IL-10, and TGF- β 1) were measured under fasting conditions. Bacterial diversity of gut microbiota was also quantitated by real-time PCR using 16S rRNA gene-based specific primers.

Results

Average value of blood sugar (P : 0.0184), insulin, HOMA-IR, TGF- β 1, IL-6, IL-1 β , IFN γ , IL-10 ($P_{\text{for each}} < 0.001$), phylum *Actinobacteria* (OR: 1.5, P : 0.032), *Firmicutes* (OR: 0.6 P : 0.058), and *Akkermansia muciniphilae* (OR: 0.4, P : 0.053) was significantly different in diabetic vs. non-diabetic obese patients but was not significantly different in hypothyroid vs. non-hypothyroid obese patients. Average value of *Bifidobacterium*, *Bacteroidetes*, *Prevotella* spp., *Bacteroides fragilis* group, *Clostridium* cluster IV, *Roseburia* spp., and *Firmicutes* to *Bacteroidetes* ratio (F/B) was not significantly different between these groups either in crude or adjusted models.

Conclusion

While there are some associations between serum biomarkers or bacterial diversity with diabetes prediction in obese patients, this prognostication is less likely in obese patients with hypothyroid. Further investigation is warranted in the application of identified preclinical biomarkers in the diagnosis of diabetes or hypothyroid in obese patients.

Introduction

Based on the World Health Organization (WHO) definition, extreme fat accumulation in people with BMI (weight (kg) divided by square of height (meter)) ≥ 30 is described as obesity. Obesity or overweight can remarkably influence the quality of life in the obese population [1].

Genetic determinants and environmental factors including an unhealthy and sedentary lifestyle, and high consumption of fattening foods have been attributed to an increased rate of obesity in the world [2, 3].

The global prevalence rate of overweight and obesity has doubled since 1980 affecting nearly one-third of the people [2]. The prevalence of obesity and severe obesity (having a greater risk of health conditions) has alarmingly increased from 1999 to 2018 in the United States [4]. It is projected that 18% of male and 21% of the female population will experience obesity by 2025 worldwide [5].

Severe obesity is implicated in the development of many medical conditions such as cardiovascular diseases, diabetes, hypothyroidism, hypertension, and cancers [2]. Recently, many studies have investigated the concomitance of diabetes mellitus and hypothyroidism in the obese population [6, 7].

According to the latest report from the International Diabetes Federation (IDF), the number of people with diabetes is expected to rise from 425 million in 2017 to 600 million with a 366 mortality rate by 2030 [7, 8].

An insufficient level of thyroid hormones released by the thyroid gland is characterized as hypothyroidism and has been linked with noticeable consequences including low metabolism rate and increased incidence of obesity [6, 9].

The gastrointestinal tract is the natural habitat of a wide variety of bacterial communities in which four main bacterial phylum *Firmicutes*, *Bacteroidetes*, *Actinobacteria*, and *Proteobacteria* are among the most dominant bacterial population [10]. The human gastrointestinal microbiota, also known as gut microbiota (GM) is estimated to be $\sim 10^{13}$ – 10^{14} microbial cells among which, *Bacteroidetes* and *Firmicutes* account for 90% of the total bacterial species [3].

As a result, the overall wellbeing of humans has been associated with a healthy and normal GM, which contributes to the maintenance of gastrointestinal function, host metabolism, and immune homeostasis. In contrast, human and animal models have shown that the unbalanced GM has been involved in the development of many human disorders including metabolic complications such as obesity and diabetes [3, 11].

Based on previous investigations, there is a reciprocal interaction between the development of metabolic complications (fluctuation in hormonal and cytokine levels) and GM in obese patients [7, 11, 12]. However, stool samples from many previous studies were evaluated using the amplicon sequencing of the 16S gene, which does not provide quantitative calculations of bacterial population and, therefore, could possibly hinder efforts to quantitatively evaluate the gut microbiota. To obtain this level of understanding, we evaluated the gut microbiota using 16S gene qPCR and serum biomarkers of diabetic and hypothyroid obese patients. Such information can provide clinically valuable information in the diagnosis and improvement of lifestyle, and therapeutical approaches in the affected population.

Materials And Methods

Ethics Statement

All procedures performed in this study involving human participants were in accordance with the principles of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the participants were thoroughly informed about the study and procedures before signing consent forms. Participants were

assured of anonymity and confidentiality. The Research Ethics Committee of the Pasteur Institute of Iran, Tehran, approved this study (Approval ID: IR.PII. REC.1397.029).

Patient Population

In this hospital-based case-control study, 120 obese patients presenting to the Department of Surgery in Shariati Educational Hospital (Tehran, Iran) with a clinical diagnosis of diabetes or hypothyroidism (18 with diabetes, 23 with hypothyroidism, and 79 without either diabetes or hypothyroidism as a control group) who were candidates for laparoscopic sleeve gastrectomy (LSG) enrolled from September 2018 to January 2020. The inclusion criteria were: (1) age limit from 30 to 50 years; (2) body mass index (BMI) ≥ 35 kg/m²; (3) without dairy allergy; (4) no infectious disease; (5) no use of antibiotic, probiotic, and medication affecting the findings over the last 6 months prior to the enrollment; (6) no gastrointestinal disease; and (7) non-pregnant/non-lactating women. Our exclusion criteria include: (1) recent illnesses (i.e. one month prior to the enrolment or less), (2) genetic or psychotic disorders, and (3) psychoactive drug misuse. Also, obese control subjects (n = 79) were selected at random among candidates for LSG, and subjects with a current or past diabetic and the hypothyroid syndrome were excluded from the control group.

Collection of Clinical Data

Each patient was provided with a standardized questionnaire regarding socio-demographic, lifestyle, anthropometric (height, weight, and BMI), medical treatments, and underlying medical history.

Hypothyroidism was diagnosed by a physician according to increased serum thyroid-stimulating hormone (TSH) level [9]. Diabetes mellitus was diagnosed according to the International Diabetes Federation (IDF) criteria [13].

Sample Collection and DNA Extraction

Stool samples were collected in a hermetically sealed sterile container and placed into the freezer immediately at -80 °C until DNA extraction. Total bacterial DNA was extracted from feces samples using the QIAamp DNA Stool Mini Kit (QIAGEN, GmbH, Hilden, Germany) following the manufacturer's instructions. DNA purity and quantity were evaluated using a Nanodrop spectrophotometer (BioTek Instruments, Inc., Winooski, VT, USA) and stored at -20 °C until processed.

Quantitative Real-Time Polymerase Chain Reaction (qPCR)

Bacterial diversity of extracted DNA from feces samples was evaluated using quantitative real-time PCR with 16S rRNA gene-based specific primers (Metabion, Germany, Table 1). DNA amplifications were performed in a final volume of 20 µL containing 10 µL 2× QPCR Green Master Mix HRox (Biotechrabbit GmbH, Hennigsdorf, Germany), 5 µM of each primer and 2 µL of target DNA. Amplification reactions were performed in a thermal cycler (StepOne™ Real-Time PCR System, Applied Biosystems, USA) under following conditions: one cycle of initial denaturation at 95°C for 3 min, 40 cycles of 95°C for 15 s, and annealing temperature suitable for each primer pair for 30 s. Standard curves were made for each run using

10-fold serial dilutions of known concentration of bacterial genomic DNA (Phylum *Proteobacteria*) and then, the copy number of the 16S rRNA gene for each species calculated using the following equation:

Number of copies = (DNA concentration (ng/μl) x [6.022 x 10²³]) / (length of template (bp) x [1x10⁹] x 650). Also, qPCR products were visualized in an agarose gel electrophoresis (Figure 1).

Biochemical and Cytokine Evaluation

Biochemistry and cytokine parameters were detected in fasting peripheral venous blood obtained from patients. Fasting blood sugar (FBS), triglycerides (Tg), cholesterol (Chol), low-density lipoprotein (LDL), high-density lipoprotein (HDL), aspartate aminotransferase (SGOT), alanine aminotransferase (SGPT), blood urea nitrogen (BUN), creatinine (CRE), and alkaline phosphatase (ALP) were measured by colorimetric enzymatic assays (COBAS MIRA® Plus).

Inflammatory cytokines (IL-6, IL-1β, and TNF-α), anti-inflammatory cytokines (IL-10 and TGF-β 1), insulin, and glucagon were analyzed using ELISA kits (Abcam, Cambridge, UK). Blood insulin was used to calculate the insulin resistance, as defined by the equation homeostasis model assessment (HOMA-IR) = fasting insulin (μU/mL) x fasting glucose (mmol/L) / 22.5. Each measure was evaluated in duplicate and the median of the two measurements was reported.

Statistical Analysis

Categorical variables were compared between diabetic/hypothyroid obese patients and the control group using Fisher's Exact Test. To check for normality Kolmogorov–Smirnov was used. To compare the association between diabetic/hypothyroid obese patients and the control group, The Kruskal Wallis test was used. Colony-forming unit (CFU) of the *Bacteroides fragilis* group, *Akkermansia muciniphilae*, *Clostridium* cluster IV, *Roseburia* spp., and *Prevotella* spp. were analyzed in log10 scale. Binary logistic regression analysis was used to estimate the effect of each unit increase in bacterial abundance to the chance of diabetes/ hypothyroidism, either in crude and adjusted models. In the adjusted model, best-fitting model was selected using the backward method.

Results

Subject Characteristics

In this study, 18 diabetic, 23 hypothyroid, and 79 control group with an average age of 37± 6.3 years old were included. In the diabetic group, 13 (76.5 %) were females. Patients had a median age of 44 years, with a mean age of 42.5 years (±7.1 years ranging from 32 to 50 years) and BMI mean of 42.6 ± 3.9 kg/m². In Subjects with hypothyroid 19 (82.6%) were female. The median age was 36 years, with mean age of 36.1 years (±4.9 years ranging from 30 to 49 years) and BMI mean of 42.7 ± 6.8 kg/m² (Table 2).

The mean age group of diabetic patients was significantly higher than the control group (*P*= 0.0054). This association, however, was not statistically significant in hypothyroid patients (*P*= 0.7799). There was no

significant difference in the distribution of other demographic, anthropometric, and clinical characteristics between diabetes/hypothyroidism and the control group (Table 2).

Biochemical and Cytokine analysis

No significant difference between biochemical indices of diabetic/hypothyroid patients and the control group was identified, except for fasting blood sugar ($P= 0.0184$), glucagon ($P< 0.001$), insulin ($P< 0.001$), and HOMA-IR ($P< 0.001$) which was significantly higher in diabetic patients compared to the control group (Table 3).

The mean value of TGF- β 1, IL-6, IL-1 β , and IFN γ was significantly higher in diabetic obese patients compared to the non-diabetic and non-hypothyroid obese patients (i.e, the control group; $P< 0.001$). Moreover, opposite result was observed for IL-10 where its mean value was significantly lower in diabetic patients compared to the control group ($P< 0.001$). None of the cytokines showed a significant difference between hypothyroid and the control group (Table 3). Significant associations between serum biomarkers and gut microbiota are presented in Supplementary File 1.

Fecal microbiota analysis

In diabetic patients, the mean value of phylum *Actinobacteria*, *Firmicutes*, and *Akkermansiamuciniphilae* was significantly different compared to the control group, however, none of the investigated bacterial communities showed significant increase/decrease in hypothyroid patients when compared to the control group (Table 4).

The crude logistic analysis showed that each unit increase in the concentration of phylum *Actinobacteria* is associated with a 1.5 fold increased chance of being diabetic compared to the control group ($P= 0.032$). Adjustment for the confounding effect of blood sugar and other bacterial population increased the strength of this association up to 2.4 folds. Other bacterial populations showed a slight protective effect on diabetes in a way that their mean concentration was slightly lower in diabetic patients compared to the control group. However, this association was only significant for the effect of *Firmicutes* ($P= 0.058$) and *Akkermansiamuciniphilae* ($P= 0.053$). Adjustment for confounding variables also did not change the direction of these associations (Figure 2, Table 4). An increased concentration of the phylum *Actinobacteria* was also associated with an increased chance of hypothyroidism compared to the control group. This association was not statistically significant either in crude or adjusted models. Other bacterial species also did not show significant effect on the likelihood of hypothyroidism, either in crude or adjusted models (Figure 2, Table 4).

Discussion

Numerous factors are closely related to the development of obesity such as high-calorie diets, lack of physical activities, changes in GM and serum biomarkers, and other environmental, genetic, and socioeconomic factors [3]. In this study, we have analyzed GM relative abundance and serum biomarkers of obese patients diagnosed with diabetes and hypothyroid disorder and determined its relationship compared to the control group.

The present study demonstrates that mean age group of diabetic patients was significantly higher than the control group. Although it might not be likely to describe an exact role of age for the onset of DM but, older adults greatly are at high risk for the development of condition.

Our results indicated that DM plays a critical role in alteration the levels of certain metabolites and the abundance of several microbial phylum and species. In this regrd, the FBS, glucagon, insulin, and HOMA-IR levels were found to be significantly elevated in obese diabetics compared to obese non-diabetics. The excess weight, the high serum glucose, and the decreasing of insulin sensitivity could explain these changes [18].

According to our findings the plasma levels of TGF- β 1, IL-6, IL-1 β , and IFN γ were markedly elevated in diabetic obese patients rather than the control group. Most of the previous studies have focused on the elevated cytokines and their role in obesity and metabolic syndrome [19–22]. In fact, inflammatory markers in combination with other factors can be considered as a predictor for the development of obesity and its related co-morbidities. Moreover, elevated serum concentrations of IFN γ can lead to the impairment of insulin sensitivity, suggesting a potential role of IFN γ as a link between the DM and insulin resisance in diabetic obese patients [23]. On the other hand, serum levels of circulating IL-10 were significantly lower in the obese with diabetes rather than the control group. Similar findings were reported that subjects with low IL-10 production capacity have an increased risk of MS such as type 2 diabetes mellitus [24, 25]. Another study shows contrast result that IL-10 level increased in obese subjects with MS compared to obese subjects without MS to inhibit continued pro-inflammatory cytokine production [26].

We revealed in this research that DM may influence compositional alterations in the gut microbiota community of obese patients that mostly apparent at both phylum and species levels. In fact, our investigations demonstrated that phylum *Actinobacteria*, *Firmicutes*, and *Akkermansia muciniphilae* was significantly different in diabetic obese patients than the control group. This could be due to several factors, including effect of disease and medications, diversity and complexity regarding to geographic origin, lifestyle of the host, and behavioral patterns. In addition, the regression analysis showed that the each unit increase in the concentration of phylum *Actinobacteria* was related with increased chance of being diabetic rather than the control group. This observation is in agreement with the documents obtained for overweight individuals by Turnbaugh and colleagues [27], though contradict with other studies [28]. We also observed a protective effect for *Firmicutes* and *Akkermansia muciniphilae* in diabetic patients rather than the control group. In this respect, some studies have pointed to a close relationship between the composition of intestinal microbiota and diabetes [28]. In agreement with our findings, a study shows that *Akkermansia muciniphila* has the ability to reduces obesity and improve glucose intolerance and insulin resistance in diabetic patients [29]. Moreover, previous studies have been reported decreased levels of *Firmicutes* in diabetic group [28]. The intestinal qPCR analysis revealed an increased abundance of Phylum *Actinobacteria* in the hypothyroid group compared with the control group. Although, this association however was not statistically significant but this finding suggest the highlighting influence of the microbiota on the thyroid disorders. It has been shown that intestinal microbiota play a role in autoimmune thyroid diseases (AITD) via modulating the immune system and host metabolism [30]. In conclusion, future adequately powered human studies are

needed to deeply understand the interactions between the gut microbiota and serum biomarkers and their impact on the diabetes and hypothyroid disorder.

Declarations

Ethics approval and consent to participate

All procedures performed in this study involving human participants were in accordance with the principles of the 1964 Helsinki declaration and its later amendments or comparable ethical standards. All the participants were thoroughly informed about the study and procedures before signing consent forms. Participants were assured of anonymity and confidentiality. The Research Ethics Committee of the Pasteur Institute of Iran, Tehran, approved this study (IR.PII. REC.1397.029).

Informed consent

All patients were provided with written informed consent for the use of personal information in manuscripts. All patients were provided with informed consent before beginning the study.

Consent for publication:

Not applicable

Availability of data and materials:

All data generated or analyzed in this study are included in the present article.

Competing interests:

The authors have no conflicts of interest.

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Authors' Contribution:

MT and SE design the study and data analysis. FSH, SDS, GM and FE data collecting and writing paper, and Investigation, Writing-review & editing. ARS and SB conceptualization, Investigation, Formal analysis, Writing-review & editing. All co-authors commented on the manuscript. All authors read and approved the final manuscript.

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Tables

Table 1. Specific primers used to amplify different bacterial groups and species of gut microbiota based on the 16S ribosomal RNA genes.

Target	Primer sequence (5'→3')	Approximately amplicon size (bp)	Annealing temp (°C)	Reference
<i>Akkermansia muciniphila</i> F	CAGCACGTGAAGGTGGGGAC	329	60	(14)
<i>Akkermansia muciniphila</i> R	CCTTGCGGTTGGCTTCAGAT			
Phylum <i>Actinobacteria</i> F	GCGKCCTATCAGCTTGTT	333	62	(12)
Phylum <i>Actinobacteria</i> R	CCGCCTACGAGCYCTTTACGC			
<i>Prevotella</i> spp. F	CACCAAGGCGACGATCA	283	60	(14)
<i>Prevotella</i> spp. R	GGATAACGCCYGGACCT			
<i>Roseburia</i> spp. F	TACTGCATTGGAACTGTCG	230	59	(14)
<i>Roseburia</i> spp. R	CGGCACCGAAGAGCAAT			
<i>Clostridium</i> cluster IV F	ACAATAAGTAATCCACCTGG	312	56	(15)
<i>Clostridium</i> cluster IV R	CTTCCTCCGTTTTGTCAA			
<i>Bacteroides fragilis</i> group F	CTGAACCAGCCAAGTAGCG	230	56	(14)
<i>Bacteroides fragilis</i> group R	CCGCAAACCTTTCACAACTGACTTA			
<i>Bacteroidetes</i> F	AAACTCAAAGAATTGACGG	196	61	(16)
<i>Bacteroidetes</i> R	GGTAAGGTTCTCGCGCTAT			
<i>Firmicutes</i> F	TGAAACTYAAGGAATTGACG	148	60	(16)
<i>Firmicutes</i> R	ACCATGCACCACCTGTC			
<i>Bifidobacterium</i> F	CTCCTGGAAACGGGTGG	550	55	(17)
<i>Bifidobacterium</i> R	GGTGTTCTTCCCGATATCTACA			
Phylum <i>Proteobacteria</i> F	CATTGACGTTACCCGCAGAAGAAGC	195	60	(14)
Phylum <i>Proteobacteria</i> R	CTCTACGAGACTCAAGCTTGC			

F: Forward; R: Revers; bp: base pair

Table 2. Demographic, anthropometric, and clinical characteristics of obese patients with diabetes mellitus or hypothyroidism and the control group[§]

Characteristic	Group			P value	
	Diabetic	Hypothyroid	Control		
	n=17	n=23	n=79	DM vs. Control	Hypothyroid vs. Control
Age (mean ± SD)	42.5 ± 7.0	36.0 ± 4.8	36.7 ± 6.1	0.005*	0.779*
Sex n (%)					
Female	13 (76.4)	19 (82.6)	57 (72.1)	0.919**	0.515**
Male	4 (23.5)	4 (17.3)	22 (27.8)		
Marital status n (%)					
Single	3 (17.6)	6 (26.0)	24 (30.3)	0.686**	0.686**
Married	14 (82.3)	17 (73.9)	52 (65.8)		
Divorced	0 (0)	0 (0)	3 (3.8)		
Educational level n (%)					
Some school	2 (11.7)	8 (34.7)	2 (2.5)	0.307**	0.772**
Diploma	7 (41.1)	15 (65.2)	35 (44.3)		
University level	8 (47.0)	0 (0)	42 (53.1)		
Job n (%)					
Government's employee	4 (23.5)	11 (74.8)	40 (50.6)	0.405**	0.937**
Housekeeper	11 (64.7)	10 (43.4)	30 (37.9)		
Self-employee	2 (11.7)	2 (8.7)	9 (11.3)		
BMI (mean ± SD)	42.5 ± 3.8	42.7 ± 6.7	43.5 (5.3)	0.451*	0.720*
Fatty liver					
Yes	5 (29.4)	5 (21.7)	11 (13.9)	0.311**	0.314**
No	12 (70.5)	18 (78.2)	68 (86.0)		

- The control group in this study was selected among non-diabetic and non-hypothyroid obese individuals.
- * Student's t-test. ** Fisher's exact test

Table 3. Biochemical indices and cytokines in obese patients with diabetes mellitus or hypothyroidism and control group^S

Characteristic	Group			Pvalue*	
	Diabetic n=17	Hypothyroid n=23	Control n=79	DM vs. Control	Hypothyroid vs. Control
FBS (mg/dl)	148.1 ± 35.3	104.6 ± 27.6	106.5 ± 32.6	0.0184	0.6868
Tg (mg/dl)	210.9 ± 138.0	144.9 ± 60.3	157.7 ± 73.6	0.3902	0.4653
SGPT (iu/l)	25.2 ± 11.8	23.7 ± 21.3	28.9 ± 22.7	0.2170	0.2505
SGOT (iu/l)	28.1 ± 14.5	26.1 ± 16.5	25.3 ± 14.9	0.7870	0.6560
ALP (iu/l)	184.7 ± 66.7	154.6 ± 52.0	165.5 ± 55.0	0.1284	0.8136
LDL (mg/dl)	87.0 ± 23.3	86.7 ± 24.3	95.5 ± 28.0	0.2517	0.3455
BUN (mg/dl)	13 ± 2.9	12.0 ± 3.0	12.0 ± 3.2	0.5271	0.7188
HDL (mg/dl)	39.8 ± 7.0	43.0 ± 11.8	41.0 ± 8.9	0.5663	0.2796
Cre (mg/dl)	1.3 ± 0.3	1.2 ± 0.3	1.3 ± 0.3	0.5271	0.7188
Urea (mg/dl)	27.8 ± 6.2	25.8 ± 6.4	26.0 ± 6.9	0.5271	0.7188
Glucagon (pg/ml)	135.9 ± 6.9	111.6 ± 19.3	103.0 ± 15.7	<0.001	0.2165
Insulin (pg/ml)	25.1 ± 1.9	17.5 ± 5.3	17.0 ± 3.4	<0.001	0.5904
HOMA-IR	9.7 ± 2.9	4.6 ± 2.1	4.4 ± 1.8	<0.001	0.5762
TGF-β 1 (pg/ml)	5367.1 ± 412.2	4321.2 ± 1095.0	4105.1 ± 1069.9	<0.001	0.8642
IL-6 (pg/ml)	7.1 ± 1.3	4.8 ± 1.8	4.8 ± 1.1	<0.001	0.0943
IL-1β (pg/ml)	62.3 ± 21.6	46.4 ± 19.6	42.6 ± 14.7	<0.001	0.9545
IFNγ (pg/ml)	67.2 ± 11.8	50.4 ± 15.6	48.9 ± 12.2	<0.001	0.8791
IL-10 (pg/ml)	21.4 ± 3.7	38.3 ± 9.8	39.6 ± 6.5	<0.001	0.2430

- The control group in this study was selected among non-diabetic and non-hypothyroid obese individuals.
* *P* values are generated using Kruskal Wallis test. Data are expressed as mean ± SD; SGOT: Serum Glutamate-Oxaloacetate Transaminase; FBS: Fasting Blood Sugar; HbA1c: Hemoglobin A1c; HOMA-IR: Homeostatic Model Assessment of Insulin Resistance; Tg: Triglyceride; Chol: Cholesterol; HDL: High-Density Lipoprotein; LDL: Low-Density Lipoprotein; BUN: Blood Urea Nitrogen; Cre: Creatinine; SGPT: Serum Glutamic-Pyruvic Transaminase; SGPT: Serum Glutamic-Pyruvic Transaminase; ALP: Alkaline Phosphatase; IL-1 β Interleukin-1 β ; IL-6: Interleukin 6; IL-10: Interleukin 10; TGF- β 1: Transforming Growth Factor Beta 1; IFN γ : Interferon Gamma.

Table 4. Crude and adjusted odd ratio of the association between gut microbiota and risk of diabetes mellitus or hypothyroidism in obese patients

	DM vs. Control				Hypothyroid vs. Control			
Variables	OR _{Crude} (95% CI)	P value	OR Adjusted (95% CI)	P value	OR Crude (95% CI)	P value	OR Adjusted (95% CI)	P value
Phylum <i>Actinobacteria</i> (ng/ul)	1.5 (1.1, 2.1)	0.032	2.4 (1.1, 5.2)	0.022	1.1 (0.8, 1.3)	0.245	1.1 (0.8, 1.4)	0.486
<i>Bifidobacterium</i> (ng/ul)	1.0 (0.7, 1.4)	0.982	0.8 (0.5, 1.5)	0.627	1.1 (0.8, 1.3)	0.334	1.0 (0.8, 1.4)	0.536
<i>Firmicutes</i> (ng/ul)	0.6 (0.4, 1.0)	0.058	0.8 (0.3, 2.0)	0.732	1.0 (0.7, 1.3)	0.987	1.0 (0.7, 1.5)	0.618
<i>Bacteroidetes</i> (ng/ul)	0.9 (0.6, 1.4)	0.704	0.9 (0.4, 1.9)	0.880	0.9 (0.7, 1.2)	0.839	1.0 (0.7, 1.3)	0.924
<i>Prevotella</i> spp. (copy/μl) CFU	1.2 (0.1, 34.8)	0.913	1.0 (0.9, 1.0)	0.126	0.9 (0.0, 1.3)	0.078	0.9 (0.9, 1.0)	0.383
<i>Bacteroides fragilis</i> group (copy/μl) CFU	0.6 (0.1, 2.6)	0.481	1.0 (0.9, 1.0)	0.552	1.2 (0.1, 8.2)	0.834	0.9 (0.9, 1.0)	0.772
<i>Akkermansia muciniphilae</i> (copy/μl) CFU	0.4 (0.1, 1.0)	0.053	1.0 (0.9, 1.0)	0.957	0.9 (0.4, 2.6)	0.982	1.0 (0.9, 1.0)	0.925
<i>Clostridium</i> cluster IV (copy/μl) CFU	0.01 (0.0, 1.9)	0.086	0.9 (0.9, 1.0)	0.202	0.9 (0.0, 42.2)	0.969	0.9 (0.9, 1.0)	0.727
<i>Roseburia</i> spp. (copy/μl) CFU	0.1 (0.0, 1.4)	0.086	1.0 (0.9, 1.0)	0.375	0.4 (0.9, 1.0)	0.251	0.9 (0.9, 1.0)	0.469
F/B ratio	0.4 (0.1, 3.8)	0.448	0.2 (0.1, 3.9)	0.290	0.9 (0.2, 4.1)	0.915	0.6 (0.9, 3.7)	0.550

OR: Odds Ratio; CI: Confidence Interval; DM: Diabetes Mellitus; F/B ratio: *Firmicutes* to *Bacteroidetes* ratio; CFU: Colony-forming unit

Figures

Figure 1

qPCR products of 16S rRNA associated genes in detected bacterial groups and species of gut microbiota.

Figure 2

Abundance of gut microbiota (mean) in obese patients with diabetes mellitus or hypothyroidism and the control group. The CFU of *Bacteroides fragilis* group, *Akkermansia muciniphilae*, *Clostridium* cluster IV, *Roseburia* spp., and *Prevotella* spp. are presented in logarithmic scale. Error bars represent standard deviation of the mean. *control group: non-diabetic and non-hypothyroid obese individuals. DM: Diabetes Mellitus. F/B ratio: Firmicutes to Bacteroidetes ratio.

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