Metabolically Healthy Obese Children and the Role of the Gut Microbiota

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Research

Keywords: metabolically healthy obese, children, 16s rRNA, gut microbiota

DOI: https://doi.org/10.21203/rs.3.rs-127205/v1

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Abstract

Background The term “metabolically healthy obese (MHO)” denotes a hale and salutary status, yet this connotation has not been validated in children, and may, in fact, be a misnomer. As pertains to obesity, the gut microbiota has garnered attention as conceivably a nosogenic or, on the other hand, protective participant.

Objective This study explored the characteristics of the fecal microbiota of obese Chinese children and adolescents of disparate metabolic status, and the associations between their gut microbiota and circulating proinflammatory factors, such as IL-6 and TNF-α, and a cytokine up-regulator and mediator, leptin.

Results Based on weight and metabolic status, the 86 Chinese children (ages 5-15 years) were divided into three groups: metabolically healthy obese (MHO, n=42), metabolic unhealthy obesity (MUO, n=23), and healthy normal weight controls (Con, n=21). In the MUO subjects, the phylum Tenericutes, as well as the alpha and beta diversity, were significantly reduced compared with the controls. Furthermore, Phylum Synergistetes and genus Bacteroides were more prevalent in the MHO population compared with controls. For the MHO subgroup, Spearman’s correlation analysis revealed that serum IL-6 positively correlated with genus Paraprevotella, and leptin correlated positively with genus Phascolarctobacterium and negatively with genus Dialister (all p<0.05).

Conclusion Dysbiosis of gut microsystem prevails in the MHO cohort, and the abundance of some metabolism-related bacteria associates with the degree of circulating inflammatory compounds. As for a role in the etiology or facilitation of obesity- or perhaps vice versa- this childhood microbial imbalance awaits long-term, longitudinal investigation.

Introduction

The global epidemic of childhood obesity, and the accompanying rise in the prevalence of endocrine, metabolic, and cardiovascular comorbidities, is perhaps the most impactful and ubiquitous public health disorder of the modern world [1]. In the context of this pandemic, a distinct subgroup of youth with obesity who are devoid of metabolic disturbances- so-called “metabolically healthy obese” (MHO) - have been identified. Obesity notwithstanding, by definition MHO children retain a favorable metabolic profile, with preserved insulin sensitivity along with normal blood pressure, glucose homeostasis, lipids, and liver enzymes. Moreover, their hormonal, inflammation, and immune profiles are seemingly impervious to obesity [2]. First described in obese adults, the MHO phenotype has also been extensively studied in young people with obesity [2]. Arguably, MHO may be a transitional stage to the far more common, more high-risk, conventional cardio-metabolic obese phenotype. Regardless of the aforesaid normal biochemical characteristics of MHO, the risk for cardiovascular disease persists since the MHO phenotype may be unstable, thereby transitory. [3, 4].
Among the non-genetic factors associated with obesity, the gut microbiota has garnered attention as an obesity regulator given the robust correlations in animal studies between gut microbiota and body weight. Obese individuals, whether adults or children, have increased abundance in *Firmicutes* in concert with decreased in *Bacteroidetes* [5, 6]. The distinctive gut bacterial flora prevalent in obese subjects is recognized as promoting an unhealthy metabolic obese (MUO) phenotype with attendant comorbidities, such as increased endotoxemia, intestinal and systemic inflammation, as well as insulin resistance. An altered gut microbiota has been implicated in obesity and type 2 diabetes mellitus (T2DM) insofar as a decrement in certain species and gene richness have been linked to adiposity, dyslipidemia, and insulin resistance [7]. Hence, the clinical repercussions aside, it is plausible that differences in the gut microbiota could dictate whether an obese child is metabolically fit (MHO) or not (MUO) [8, 9].

Firstly, this study examined the metabolic heterogeneity of obese children as it relates to the composition of the bacterial flora of the gut. And, as a secondary end point, identify metabolic-specific bacteria which associate with serum inflammatory factors incriminated in obesity comorbidities.

**Results**

1. **Study participants**

Based on weight status, the metabolically stable cohort (MH) subjects (n=63) were subdivided as MHO (n=42) or Con (n=21). Recall that, by definition, the MH refers to a merger of the Con and MHO cohorts ∴ MH= Con and MHO.

The age of the 86 participatees ranged from 5.5 to 14.3 years, with a mean of 9.76 ±1.93 years. There were 65 obese children, of whom 23 were MUO and 42 were MHO. The BMI of other 23 children were normal. Age, BMI, BMI-Z, WHR, SBP, TG and LDL-c in the MUO group were significantly higher than the MH group, and HDL-c in the in the MUO group were significantly lower than the Con and MHO children (all p<0.05, Table 1).

The BMI, BMI-Z, WHR, WHtR, SBP, DBP, TG and LDL-c were significantly higher in the MHO group than the Con children, and HDL-c in the MHO group were significantly lower than the Con group (all p <0.05). There was no statistical difference in age, gender, FPG and fasting TC between MHO and Con (all p>0.05, Table 1).

2. **Microbiota Profiles in different metabolic status subjects**

A total of 918,578 sequencing reads were obtained from 86 fecal samples, with an average value of 10,681 counts per sample. We identified an overall of 146 OTUs, among which 136 OTU with ≥2 counts, and they were grouped in 9 phylum and 38 families.

(1) **Abundance profiling in different metabolic status subjects**
Grouping OTUs at phylum level, and applying the Mann-Whitney U test on the relative abundances of phyla for the two groups, the relative abundances of phylum *Tenericutes* was more prevalent in the MH subjects (recall that MH=Con and MHO) compared to the MUO group \( (p = 0.006, \text{Table S1 and Figure 1a}) \).

On OTUs at the genera level, by Mann-Whitney U-test, including all the genera (merging small taxa with counts<10), we identified that genera *Anaerostipes*, *Alistipes*, *Desulfovibrio*, *Fusobacterium*, *Gemmiger*, *Odoribacter*, *Oscillospira* and *Parabacteroides* were more prevalent in the two metabolically healthy cohorts (MH) versus MUO children, yet the genus *Dorea* was more prevalent in MUO \( (p < 0.05; \text{Figure 1b, Table 2}) \).

### (2) Alpha- and beta-diversity in different metabolic status subjects

To assess the overall differences of microbial community structures in MH and MUO subjects, we measured ecological parameters based on alpha-diversity. The alpha-diversity analysis showed significantly higher diversity in MH in comparison to MUO participants \( (p<0.05, \text{Figure 2 a,b, Table S2}) \).

To determine the differences between microbial community profiles in MH and MUO subjects, we calculated beta-diversity. By Distance method Bray-Curtis dissimilarities PCoA analysis, the gut microbiota samples from MH were clustered together and separated partly from the MUO group. Upon analysis, metabolism explained 19.8% of the variance in microbiota composition \( (P = 0.038, \text{Figure 2c, Table S3}) \).

### (3) Bacterial taxa differences in different metabolic status subjects

We next used LEfSe analysis to identify bacteria in which the relative abundance was significantly increased or decreased in each phenotypic category. The MH subjects had members of the phylum *Tenericutes*, class *Deltaproteobacteria*, *Mollicutes*, order *Desulfovibrionales*, RF39, family *Christensenellaceae*, *Odoribacteraceae*, *Porphyromonadaceae*, *Ruminococcaceae*, genera *Anaerostipes*, *Oscillospira*, *Odoribacter*, *Gemmiger*, *Parabacteroides*, *Alistipes*, that were significantly higher than MUO subjects. Furthermore, the MUO subjects had members of the genus *Fusobacterium* that were significantly higher than the MH subjects \( (\text{all } p<0.05, \text{Figure 3a, b}) \).

### 3. Microbiota Profiles in obese subjects with different metabolic status

#### (1) Abundance profiling in obese subjects with different metabolic status

Grouping OTUs at phylum level, and applying the Mann-Whitney U test on the relative abundances of phyla for the MHO and MUO groups, the relative abundance of phylum *Tenericutes* was more prevalent in the MHO group compared to the MUO group \( (p = 0.027, \text{Table 3 and Figure 1c}) \).

On OTUs at the genera level, by Mann-Whitney U analysis, including all the genera (merging small taxa with counts<10), we identified that genera *Desulfovibrio*, *Parabacteroides* and *Gemmiger* were more
prevalent in MHO subjects compared to MUO subjects ($p = 0.027$, $0.040$ and $0.047$, respectively; Figure 1d).

**(2) Alpha- and beta-diversity between MHO and MUO subjects**

Regarding alpha-diversity, in both the MHO and MUO group, the analysis exposed significantly higher diversity in MHO subjects versus MUO participants (all $p <0.05$, Figure 2d,e, Table S2).

Regarding beta-diversity, by an unweighted-UniFrac method, the MHO group was lower than the MUO group ($p=0.021$, Table S3).

**(3) Bacterial Taxa Differences between MHO and MUO subjects**

LEfSe analysis showed MHO subjects had members of the phylum *Tenericutes, class Deltaproteobacteria, Mollicutes, order Desulfovibrionales, RF39, family Christensenellaceae, Odoribacteraceae, Rikenellaceae, Desulfovibrionaceae, Porphyromonadaceae, Ruminococcaceae*, genus *Gemmiger, Parabacteroides* that were significantly higher than MUO subjects (all $p<0.05$, Figure 3c, d).

4. Microbiota Profiles in MHO and Con subjects with different weight status

**(1) Abundance profiling in metabolically healthy subjects with different weight status**

Grouping OTUs at phylum level, the relative abundances of phylum *Synergistetes* was more prevalent in the MHO group compared to the Con group ($p < 0.05$, Figure 1e, Table 4).

On OTUs at the genera level, including all the genera (merging small taxa with counts<10), genera *Anaerotruncus, Bacteroides, Adlercreutzia* and *Pyramidobacter* were more prevalent in MHO subjects versus MUO subjects ($p < 0.05$; Figure 1f).

**(2) Alpha- and beta-diversity between different weight status**

Regarding alpha-diversity, the Shannon diversity index, Observed OTUs, Faith's phylogenetic diversity and Pielou's evenness based on OTU distribution did not reveal any significant difference between MHO and Con (all $p >0.05$, Table S2); also, beta-diversity did not differ significantly between these two groups. Importantly, none of the comparisons were significantly different (all $p>0.05$) after correction for multiple testing (Table S3).

**(3) Bacterial Taxa Differences in MHO and Con subjects of different weight status**

LEfSe analysis showed MHO subjects had members of the phylum *Synergistetes, class Synergistia, order Synergistales, Erysipetotrichales, family Dethiosulfovibrionaceae, genus Pyramidobacter* were significantly higher than the Con-, however, the latter had members of the family *Bacteroidaceae, genus Anaerotruncus* that were significantly higher (all $p<0.05$, Figure 3e, f).

5. Detecting Microbial Biomarkers in different metabolic status
Discriminant analysis (DA) based on univariate ANOVAs, Fisher's coefficient and leave-one-out classification were performed to define a model based on the capability of OTUs to discriminate the three groups of study participants (MHO, MUO and Con).

A DA showed that 58.1% of the original grouped subjects were correctly classified, and the canonical discriminant plot revealed partly separation among groups (Figure 4 and Table S4). Furthermore, applying a cross-validation (CV) test, we found that 54.7% of cases were correctly classified, attesting to the capability of the entire OTUs set to discriminate the three groups (Table S4).

6. Correlations between inflammatory factors and bacterial abundance

To evaluate correlations between bacteria and serum inflammatory factors (IL-6, TNF-α and leptin), Spearman's rho cut-off values were assessed, taking into account r > 0.4, r < -0.4 (p < 0.05, Table S5).

For MUO subjects, Spearman's correlation analysis revealed that IL-6 positively correlated with genus Lactococcus, TNF-α positively correlated with phylum Bacteroidetes, negatively correlated with genus Citrobacter. Leptin positively correlated with genus Eubacterium and negatively correlated with genus Faecalibacterium and Lachnospira (all p<0.05, Table S5).

For MHO subjects, Spearman's correlation analysis revealed that serum IL-6 positively correlated with genus Paraprevotella. Leptin positively correlated with phylum Bacteroidetes, Firmicutes, genus Phascolarctobacterium and negatively correlated with genus Dialister (all p<0.05).

7. Metabolic Pathway Predictions

A total of 15 KEGG pathways were generated using the composition of the fecal microbiota based on PICRUSt2 in the MH cohorts versus MUO subjects (Figure 5, Table S6). In the comparison between MHO and MUO subjects, we obtained 3 differential pathways (Figure 5, Table S7). Moreover, 11 differential metabolic patterns differentially expressed resulted in the comparison between MHO versus Con (Figure 5, Table S8).

Discussion

Recognized for decades, there is wide-ranging heterogeneity among obese individuals as to their risk for developing metabolic dysfunction and attendant complications [10]. Also well-established, and which may contribute to this metabolic heterogeneity, is the observation those with central obesity are more prone to developing T2DM and cardiovascular disease than those with peripheral obesity [11]. In this study, to indirectly address the issue of fat distribution, we found there were no significant differences in WHR and WHtR between the two obese cohorts, MHO vs MUO.

A chronic low-grade inflammation, triggered by nutrient surplus, is a constituent of obesity. Adipose-originated metabolic inflammation develops pari passu with insulin resistance and, as such, is a key element in the metabolic syndrome [12]. In this study, we found there were no significant differences in
serum IL-6, TNF-α and leptin between MHO and MUO subjects. It stands to reason that, besides these cytokines, other biochemical factors likely contribute to the metabolic diverseness in obese subjects. Or, perhaps, the concentrations of circulating compounds—such as those abovementioned—poorly reflect those found in extracellular or intracellular tissues.

Evidence can be adduced that the gut microbiota is involved in the aetiology of obesity and obesity-related complications such as nonalcoholic fatty liver disease, insulin resistance and T2DM [13, 14]. These disorders are characterized by alterations in the diversity of the gut microbiota, and the relative abundance of certain genera. And bacteria-generated metabolites, translocated from the gut across a disrupted intestinal barrier, can affect several metabolic organs, such as the liver and adipose, thereby contributing to systemic metabolic inflammation [15].

Recently, several animal studies concluded that an optimal healthy-like gut microbiota may bestow a more propitious obese phenotype [16, 17]. For instance, the abundance of Bacteroidetes and Tenericutes were closely aligned with bile acid metabolism and obesity-related inflammation in a murine model of the metabolic syndrome [18]. In our study, we corroborate this finding: reduced abundance of Tenericutes in the MUO group compared with the metabolically healthy groups (MHO and Con). Similarly, in high fat diet-induced obese mice, β-glucan favorably increases bacteria that generate butyrate (such as Anaerostipes), thereby mitigating hepatic stress and intestinal atrophy [19]. In another study, gamma-aminobutyric acid enriched rice bran ameliorated the metabolic syndrome (insulin resistance, lipids) in dietary-induced obese rats by enhancing Anaerostipes production of two salutary short-chain fatty acids, C2 butyrate and C3 propionate, and manifest both in the intestine and circulation. Finally, another benefit was a significant upturn in serum leptin and glucagon-like peptide-1 [20]. We also observed more abundance of Anaerostipes in the MH cohort, as well as the alpha and beta diversity. These results buttress the notion of dysbiosis in the gut microbiota of MUO individuals.

To characterize the gut microbiota in obese children of different metabolic status, we further analyze the MHO and the MUO subgroups. The abundance of Tenericutes was significantly reduced in the MUO group compared with the MH children, indicating that Tenericutes is related to the metabolic state, and the bacterial imbalance is independent of weight. Previously reported, the abundance of Parabacteroides was significantly decreased in obese subjects with metabolic syndrome [6], and nonalcoholic fatty liver disease [21], and negatively correlated with weight gain and leptin plasma levels [22]. And germane to our findings, both genera Gemmiger [30] and Parabacteroides [23] are gut bacteria negatively associated with obesity and disturbed host metabolism. In accordance, we found that that the fecal abundance of these bacteria was significantly higher in the MHO group compared with MUO.

The genera Parabacteroides are short-chain fatty acids (SCFAs)-producing bacteria. SCFAs are low molecular weight molecules produced from fermentation of dietary fiber or polysaccharides by gut microbiota. Absorbed by the intestinal epithelium into the blood, they can beget physiological disorders in the host, such as deranged lipid metabolism and intestinal environment imbalances [24, 25]. Furthermore,
alpha and beta diversity were significantly higher in MH subjects compared with the MUO group, again supporting the notion of dysbiosis in the unhealthy MUO population.

Notwithstanding that the gut microbiota obese individuals with metabolic syndrome may indeed unhealthy, is the gut microbiota of the MHO population really healthy? We compared the characteristic of gut microbiota in the MH population of different weights. Even though there was no significant difference in alpha and beta diversity, the relative abundances of phylum *Synergistetes* and genus *Bacteroides* were elevated in the MHO group compared to the Con children. Based on a metagenomic approach and bioinformatics analysis in obese adults, it is plausible that an abundance of the microbiota taxa *Bacteroides* could portent the evolution to T2DM [26].

Alterations in gut ecology can propel inflammatory pathways in several tissues, resulting in glucose intolerance and CVD [27, 28]. In rodents, both the dysregulation of the tandem microbiota-host metabolism of bile acids and also the bacterial production of lipopolysaccharides (i.e., endotoxemia) can beget derangements in glucose homeostasis [29, 30]. Herein, we found that, depending on the metabolic status, the serum levels of classic proinflammatory factors IL-6, TNF-α and leptin were related to the abundance of various fecal bacteria. Notably, in MHO children, serum leptin correlated positively with genus *Phascolarctobacterium* and negatively with *Dialister* – the latter genera observed with low abundance in obese children [31]. And, relevant to our findings, it is noteworthy that *Phascolarctobacterium* purportedly is a biomarker for adult T2DM [26]. As illustrated in our MHO children and the above-cited studies in humans, the gut microbiota is a marquee player in preserving normal metabolism despite obesity or, perhaps, an ephemeral protective flora destined to change with transition to MUO.

**Conclusion**

In aggregate, the MUO population had lower alpha- and beta- diversity, and lower abundance of *Tenericutes*, which were independent of weight, inferring a robust inter-relationship between gut bacterial ecology and host metabolic state. In the MHO population, phylum *Synergistetes* and genus *Bacteroides* and *Phascolarctobacterium* were more prevalent, and the abundance of some metabolism-related bacteria correlated with circulating proinflammatory factors, suggesting that dysbiosis of gut microbiota was already extant in the MHO children, conceivably a compensatory or remedial response to a surfeit of nutrients.

**Methods**

**Study population**

This study was approved by the Ethics Committee of the Fuzhou Children's Hospital of Fujian Medical University and, in all cases, informed consent was obtained.
The cross-sectional study consisted of participants managed by Fuzhou Children's Hospital of Fujian Medical University from September 2017 to March 2018. This study was limited to participants who met the following criteria: (a) ages between 5 to 15 years old, and (b) residence of Fujian province.

The exclusion criteria were as follows: any endocrine disorder, history of antibiotic therapy in the past 3 months prior to the enrollment, chronic gastrointestinal illness or use of gastro-intestinal-related medication, or diarrheal disease (World Health Organization definition) in the past one month.

**Clinical assessment**

Height and weight were measured by trained nurses. BMI-Z scores were calculated based on reference values of Li Hui et al [32]. At the end of normal expiration, waist and hip circumference were measured to the nearest 0.5 cm using standard technique with nonelastic tape. Waist circumference was measured at a point midway between the lower border of the ribs and the iliac crest, and hip circumference was measured at the widest part of the hip. A waist-to-hip ratio (WHR) was calculated by waist circumference (cm) divided by hip circumference (cm) and a waist-to-height ratio (WHtR) by waist circumference (cm) divided by height (cm).

**Laboratory examination**

All participants maintained their usual dietary pattern at least 3 days before blood sampling. After 12 h of fasting, 10 ml venous blood was drawn by registered nurses. All blood samples were stored at −80°C, and analyzed within two weeks of sampling. Serum IL-6 was measured using a commercial ELISA kit (Abcam, UK), with an 4.4% inter-assay coefficient of variation (CV). Serum TNF-α levels was measured using a commercial ELISA kit (Abcam, UK), with inter-assay and intra-assay CVs of 3.3% and 9%, respectively, and serum leptin assayed using a commercial ELISA kit (Abcam, UK), with inter-assay and intra-assay CVs of 2.4% and 2.7%, respectively. Fasting plasma glucose (FPG) and plasma lipids, including total cholesterol (TC), triglyceride (TG), high-density lipoprotein cholesterol (HDL-c) and low density lipoprotein cholesterol (LDL-c), were assayed by standard methods using specific reagents (Beckman Coulter AU5800, USA). Fasting insulin (INS) was determined by a chemiluminescent immunoassay (IMMULITE 2000, Siemens Healthcare Diagnostics Products Limited, Germany). Fecal samples were collected and processed as previously described [33].

**Definition of metabolic unhealthy**

Metabolic syndrome parameters were applied according to 2019 Expert Committees [34], and MUO was defined by the presence of at least one of the following metabolic traits: (1) FPG ≥ 5.6 mmol/L; (2) systolic blood pressure ≥ 90th percentile for gender and age; (3) fasting HDL-C< 1.03 mmol/L; and (4) fasting TG ≥ 1.7 mmol/L.

**Genomic DNA extraction and Library Construction**
The microbial community DNA was extracted and quantified as previously described [33]. Variable regions V3-V4 of bacterial 16s rRNA gene were amplified with degenerate PCR primers [33]. Libraries were qualified by the Agilent 2100 bioanalyzer (Agilent, USA). The validated libraries were used for sequencing on Illumina MiSeq platform (BGI, Shenzhen, China) following the standard pipeline of Illumina, and generating 2 × 300bp paired-end reads.

**Statistical analysis**

Statistical analyses of clinical data were performed using the Statistical Package for the Social Sciences software version 23.0 (SPSS Inc. Chicago, IL, USA). The normality of the data was tested by Kolmogorov-Smirnov test. Data are expressed as mean ± SD. Comparisons of the results were assessed using independent samples t test, Mann-Whitney U test and Kruskal-Wallis test, depending on the type of data distribution (e.g., non parametric). Comparison of rates between two groups was by chi-square. A value of $P < 0.05$ was deemed statistically significant.

Statistical analysis of 16s rRNA sequencing data were performed on alpha- and beta- diversity measurements, which was done by software QIIME2(v2019.7) [35]. Kruskal-Wallis Test was adopted for two groups comparison. Linear discriminant analysis Effect Size (LEfSe) Analysis was assessed by software LEFSE [36]. To predict metagenome functional content from 16S rRNA gene surveys, Picrust2 [37] have been applied to obtain the KEGG (Kyoto Encyclopedia of Genes and Genomes) pathways, and STAMP [38] was used to analyze the differential pathways.

**Declarations**

**Ethics approval and consent to participate**

This study was reviewed and approved by the Ethics Committee of Fuzhou Children's Hospital of Fujian Medical University, and was conducted in agreement with the Declaration of Helsinki Principles. Informed consent was obtained from all individual participants included in the study.

**Consent for publication**

Informed consent for publication was obtained from all individual participants included in the study.

**Availability of data and materials**

The original contributions presented in the study are publicly available. The raw sequence data reported in this paper have been deposited in the Genome Sequence Archive (Genomics, Proteomics & Bioinformatics 2017) in National Genomics Data Center (Nucleic Acids Res 2020), Beijing Institute of Genomics (China National Center for Bioinformation), Chinese Academy of Sciences, under accession number CRA003010 that are publicly accessible at https://bigd.big.ac.cn/gsa.

**Competing interests**
The authors declare that they have no competing interests.

**Funding**

This study was supported by Technology Innovation Team Train Project of Fuzhou Health Committee in China (2016-S-wp1), and sponsored by key Clinical Specialty Discipline Construction Program of Fuzhou, Fujian, P.R.C. (201610191) and Fuzhou Children's Medical Center (2018080310).

**Author Contributions**

XY drafted the initial manuscript; RMC conceptualized and designed the study, and reviewed and revised the manuscript; KL. M revised the manuscript; YZ and XHY collected cases; XQL did the laboratory testing.

**Acknowledgements**

The authors are grateful to all the participants.

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Tables
|                  | MUO (n = 23) | MH Total (n = 63) | MHO (n = 42) | MHN (n = 21) |
|------------------|--------------|------------------|--------------|--------------|
| Age (yr)         | 10.96 ± 1.69 | 9.32 ± 1.84*     | 9.47 ± 1.68* | 9.02 ± 2.14  |
| Male(%)          | 65.2         | 50.8             | 54.8         | 42.9         |
| BMI (kg/m²)      | 27.02 ± 2.75 | 21.80 ± 4.91*    | 24.65 ± 3.14*| 16.11 ± 1.91#|
| BMI-Z            | 2.81 ± 0.61  | 1.77 ± 1.53*     | 2.74 ± 0.60  | -0.16 ± 0.79#|
| WHR              | 0.89 ± 0.05  | 0.86 ± 0.06      | 0.88 ± 0.05  | 0.84 ± 0.06# |
| WHtR             | 0.55 ± 0.04  | 0.50 ± 0.06*     | 0.53 ± 0.04  | 0.43 ± 0.03# |
| SBP(mmHg)        | 116.45 ± 8.77| 101.52 ± 8.36*   | 105.51 ± 6.96*| 94.48 ± 5.51#|
| DBP(mmHg)        | 65.09 ± 5.72 | 62.57 ± 5.79     | 63.81 ± 6.45 | 60.38 ± 3.56#|
| FPG(mmol/L)      | 5.09 ± 0.67  | 4.87 ± 0.39      | 4.82 ± 0.38* | 4.97 ± 0.40  |
| TC(mmol/L)       | 4.54 ± 0.90  | 4.30 ± 0.62      | 4.39 ± 0.57  | 4.14 ± 0.69  |
| TG(mmol/L)       | 1.62 ± 0.99  | 0.86 ± 0.30*     | 0.93 ± 0.33* | 0.72 ± 0.19# |
| LDL-c(mmol/L)    | 2.65 ± 0.66  | 2.31 ± 0.53*     | 2.45 ± 0.48  | 2.03 ± 0.54# |
| HDL-c(mmol/L)    | 1.24 ± 0.24  | 1.58 ± 0.30*     | 1.51 ± 0.30* | 1.71 ± 0.26# |
| leptin(µg/ml)    | 2.70 ± 1.48  | 2.23 ± 1.83      | 3.10 ± 1.65  | 0.51 ± 0.35*#|
| TNF-α(pg/ml)     | 47.50 ± 25.63| 48.48 ± 18.77    | 53.43 ± 17.88| 38.59 ± 16.81#|
| IL-6(ug/ml)      | 1.76 ± 0.86  | 1.65 ± 0.93      | 1.86 ± 1.04  | 1.23 ± 0.42*#|

BMI: body mass index, BMI-Z: BMI standard deviation Z score, WHR: waist-to-hip ratios, TC: total cholesterol, TG: triglyceride, LDL-c: low-density lipoprotein cholesterol, HDL-c: high density lipoprotein cholesterol, LBP: lipopolysaccharide-binding protein.*: compared with the MUO group, p < 0.05; #: compared with the MHO group.
Table 2
The mean relative abundance of gut microbiota with significantly differences in different metabolic status at genera level

|                | MUO  | MH  | Z     | P value |
|----------------|------|-----|-------|---------|
| Anaerostipes   | 0.001| 0.001| -2.084| 0.037   |
| Odoribacter    | 0.000| 0.002| -2.122| 0.034   |
| Desulfovibrio  | 0.000| 0.003| -2.142| 0.032   |
| Alistipes      | 0.010| 0.023| -2.182| 0.029   |
| Fusobacterium  | 0.001| 0.002| -2.185| 0.029   |
| Dorea          | 0.012| 0.005| -2.288| 0.022   |
| Gemmiger       | 0.007| 0.013| -2.32  | 0.020   |
| Oscillospira   | 0.008| 0.010| -2.445| 0.014   |
| Parabacteroides| 0.007| 0.020| -2.552| 0.011   |

Table 3
The mean relative abundance of gut microbiota obese subjects with different metabolic status at phylum level

|                | MHO  | MUO  | Z     | P value |
|----------------|------|------|-------|---------|
| Actinobacteria | 0.012| 0.025| -0.783| 0.434   |
| Bacteroidetes  | 0.453| 0.371| -0.823| 0.41    |
| Firmicutes     | 0.393| 0.321| -0.919| 0.358   |
| Fusobacteria   | 0.006| 0.016| -1.494| 0.135   |
| Proteobacteria | 0.132| 0.267| -0.535| 0.593   |
| Tenericutes    | 0.003| 0.000| -2.212| 0.027   |
| Verrucomicrobia| 0.001| 0.000| -1.48  | 0.139   |
Table 4
The mean relative abundance of gut microbiota with significantly differences in obese subjects with different metabolic status at genera level

|                  | MHO | MHN | Z     | P value |
|------------------|-----|-----|-------|---------|
| **Actinobacteria** | 0.012 | 0.018 | -1.181 | 0.238   |
| **Bacteroidetes**  | 0.319 | 0.377 | -1.006 | 0.314   |
| **Cyanobacteria**  | 0.000 | 0.000 | -1.245 | 0.213   |
| **Firmicutes**     | 0.572 | 0.531 | -0.831 | 0.406   |
| **Fusobacteria**   | 0.006 | 0.014 | -0.324 | 0.746   |
| **Proteobacteria** | 0.088 | 0.057 | -1.881 | 0.060   |
| **Synergistetes**  | 0.000 | 0.000 | -1.964 | **0.050** |
| **Tenericutes**    | 0.002 | 0.002 | -1.408 | 0.159   |
| **TM7**            | 0.000 | 0.000 | -0.481 | 0.630   |
| **Verrucomicrobia**| 0.001 | 0.001 | -0.177 | 0.859   |

Figures
Figure 1

Bar chart representing Mann-Whitney U-test results on operational taxonomic units (OTUs) grouped in phyla (a, c, e) and in genus (b, d, f) of the different metabolic status groups. Each column in the plot represents a group, and each color in the column represents the percentage of relative abundance for each OTU.
Figure 2

Characterization of alpha- and beta-diversity of the gut microbiota in MH, MUO and MHO groups. The y-axes show the Shannon index (a, c) and Chao1 richness index (b, d). The x-axes show the phenotypic categories. Additional data are in Table S2. Principal coordinates analysis (PCoA) plot of MH and MUO groups (e). The plots show the first two principal coordinates (axes) for PCoA using Bray-Curtis Distance method.
Figure 3

Differential biomarkers associated with different metabolic status. A linear discriminant effect size (LeFse) analysis have been performed ($\alpha$ value = 0.05, logarithmic LDA score threshold = 2.0).
Figure 4

Canonical discriminant plot. Scatter plot of canonical discriminant analysis (DA) based on univariate ANOVA and Fisher’s coefficient applied to all OTUs of samples belonging to MHO, MUO, and Con subjects.
Figure 5

KEGGs biomarkers associated with different metabolic status.

Supplementary Files

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